

Phylogenetic relationships and population dynamics of *Calonectria*

Conrad L. Schoch



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Promoter: Prof P.W. Crous,
Co-promoters: Proff M.J. Wingfield and B.D. Wingfield

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Declaration

I the undersigned hereby declare that the work contained in this dissertation is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

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Summary

This dissertation is presented as a collection of separate publications and an amount of redundancy has thus been unavoidable. Although several species are newly described they are not effectively published and will thus be formally published in scientific journals. There were two main objectives:

- I. To investigate the variability and mating compatibility of species and populations, in order to contribute to the systematics of *Calonectria*.
- II. To identify loci that would be useful for DNA sequence comparisons in this genus and to present a reliable phylogeny of *Calonectria* and other closely related hypocrealean taxa.

In the introductory review a synopsis of the current knowledge regarding the taxonomy and life cycle of *Calonectria* and *Cylindrocladium* spp. is presented. The importance of these pathogens are noted, as well as the problems related to identifying them. Aspects regarding specific species complexes and topics are discussed in more detail in the following chapters.

The morphological and phylogenetic variation was investigated for the *Cy. candelabrum* species complex in Part 2. DNA sequence comparisons of the ribosomal 5.8S gene and flanking ITS1 and ITS2 spacers were employed in order to determine whether mating incompatibility and general morphology was supported by molecular evidence. Although only small differences were found these proved to be consistent and resulted in the recognition of *Calonectria scoparia* (anamorph *Cylindrocladium candelabrum*), and the description of three new species, namely *Calonectria pauciramosa* (anamorph *Cylindrocladium pauciramosum*), *Calonectria insularis* (anamorph *Cylindrocladium insulare*) and *Calonectria mexicana* (anamorph *Cylindrocladium mexicanum*).

The *Cylindrocladium scoparium* cultures studied in Part 3 were isolated from several hosts in the U.S.A. Isolates were mated in all combinations, and one successful mating was selected to establish whether recombination occurred. RAPD and mating type data of parental isolates and progeny confirmed *Cy. scoparium* to have a heterothallic mating system. Furthermore, to determine the phylogeny of *Cy. scoparium* with several morphologically similar *Cylindrocladium* spp., DNA

sequences of the ribosomal 5.8S gene and the flanking internal transcribed spacers (ITS), as well as part of the high mobility group (HMG) box (forming part of the *MAT-2* mating type gene) and the β -tubulin gene, were analysed. Maximum parsimony yielded concordant trees for all three data sets. These data supported the morphological and biological species concepts proposed for *Cy. scoparium* and other, similar, small-spored *Cylindrocladium* spp.

Part 4 represented an investigation into the mating compatibility and mating type distribution of populations of *Cy. pauciramosum*. This enabled the determination of the effective population for the different areas studied. A sample collected over a period of six years, reflecting a number of locations in South Africa were found have 1:1 mating type ratio, as expected in a random mating population. However, the mating type ratio was found to be significantly different in single nursery populations. In the South African nursery, the *MAT-1* mating type was dominant, while the *MAT-2* was more common in other samples obtained from nurseries in Italy and the U.S.A. This was consistent with one or more founder effects. The high percentage of hermaphrodites also suggested that recent introductions had occurred in nurseries in Italy and the U.S.A. In addition to this, DNA sequence comparisons of the β -tubulin gene was used to investigate variation below species level in *Cy. pauciramosum*. All isolates from South Africa, Australia, U.S.A. and a group from Italy had identical sequences. A second group with identical sequences were found in the Italian sample. In addition to this, variation was found between all isolates from Brazil, Colombia and Mexico. Some of these base pairs were shared between the South and Central American isolates as well as isolates of *Cy. candelabrum*. This points towards a speciation event in South or Central America.

After investigating variation below species level, this study was also expanded to generic level. In Part 5 information obtained in the preceding chapters culminated in a phylogeny of all known species in *Calonectria* and *Cylindrocladium* based on DNA sequence comparisons of the β -tubulin gene. Many clades, containing small numbers of isolates were strongly supported by bootstrap. However, relationships between these clades were often ambiguous. A number of phylogenetic placements based on DNA data did not always agree with preconceived morphological relationships. Two large groupings were evident and both contained small-spored, one-septate species. The only morphological character that correlated with DNA based phylogenies was vesicle shape of the anamorph.

Finally, in Part 6, the generic phylogeny was investigated. In order to obtain a generic phylogeny a subset of *Calonectria* species was selected, as well as isolates from other genera, closely related to *Calonectria*. All of these genera were originally described under the broad concept of *Nectria* sensu lato. A gene tree phylogeny, based on β -tubulin was presented for selected nectriaceous genera with anamorphs bearing cylindrical macroconidia. Based on molecular data and the distinct anamorph genera, new teleomorph genera were proposed for *Cylindrocladiella* (*Nectricladiella*), *Gliocladiopsis* (*Glionectria*) and *Xenocylindrocladium* (*Xenocalonectria*). *Calonectria* was also found to form a monophyletic lineage. Eight species of *Cylindrocladiella* were recognised, with two having teleomorphs in *Nectricladiella*, namely *N. camelliae* (*Ce. microcylindrica*) and *N. infestans* (*Ce. infestans*).

This study concluded that the current morphological species concepts in *Cylindrocladium* and its *Calonectria* teleomorphs can comprise several biological as well as phylogenetic species. The use of mating testers in this study was shown to provide a powerful tool to separate morphologically similar, but genetically isolated species. The biological and morphological species also agreed with the phylogenetic concepts used, but only vesicle shape were found to define phylogenetic clades. However, phylogenetic species concepts based on DNA sequences data obtained from genomic regions such as the β -tubulin and *MAT-2* genes and additional areas will become increasingly important for further taxonomic studies in *Calonectria* and related genera.

Opsomming

Hierdie studie word aangebied as 'n samevoeging van 'n aantal onafhanklike publikasies en 'n sekerre mate van oorvleueling sal dus voorkom. Alhoewel 'n aantal spesies nuut beskryf word in hierdie tesis, is hulle nie effektief gepubliseer nie, en sal dergelyke publikasie in toepaslike wetenskaplike joernale plaasvind. Die hoofdoel van hierdie studie was tweërlei:

- I. Om die varieerbaarheid en paringsvermoëns van spesies en bevolkings te ondersoek en by te dra tot die sistematiek van *Calonectria*.
- II. Om die lokusse te identifiseer wat bruikbaar kan wees vir DNA volgorde vergelykings in hierdie genus en om 'n betroubare filogenie van *Calonectria* en naby verwante spesies in die Hyporeales te genereer.

In die inleidende oorsig is die huidige kennis aangaande die taksonomie en lewensiklus van *Calonectria* en *Cylindrocladium* spp. bespreek. Die belang van hierdie spesies is aangedui, sowel as die probleme waarmee hulle geassosieer word. Punte wat van toepassing is op spesifieke spesie komplekse word later in meer detail bespreek.

Die morfologiese en filogenetiese variasie op spesie vlak word ondersoek in Deel 2. DNA volgorde vergelykings van die ribosomale 5.8S geen en die naasliggende ITS1 en ITS2 intergeniese areas was gebruik om die paringsonvermoë en morfologiese karakters te onderskryf. Dit het die herbeskrywing van 'n bestaande spesie, *Calonectria scoparia* (anamorf *Cylindrocladium candelabrum*), en die beskrywing van drie nuwe spesies, *Calonectria pauciramosa* (anamorf *Cylindrocladium pauciramosum*), *Calonectria insularis* (anamorf *Cylindrocladium insulare*) and *Calonectria mexicana* (anamorf *Cylindrocladium mexicanum*) tot gevolg gehad.

In die daaropvolgende deel was die herkombinering van *Cy. scoparium* beskou met behulp van RAPD merkers. Parings is uitgevoer en die RAPD en paringstipe data het bevestig dat hierdie spesie heterotallies is. In die tweede deel van hierdie hoofstuk was DNA volgorde vergelykings gedoen op fragmente wat verkry is van drie verskillende lokusse, die 5.8S ribosomale geen en ITS areas en dele van die *MAT-2* geen se HMG kas asook die β -tubulien geen. Hierdie data was aangewend om die filogenie van *Cy. scoparium* en ander kleinspoorvormende heterotalliese

Cylindrocladium spesies te ondersoek. Dit het die drie nuut beskryfde spesies van die vorige hoofstuk ingesluit en morfologies en biologies spesie konsepte bevestig.

Deel 4 bevat 'n ondersoek na die paringsvermoëns van *Cy. pauciramosum* op bevolkingsvlak. As gevolg hiervan kon die effektiewe bevolking in verskillende areas bepaal word. A monster wat oor 'n tydperk van ses jaar en 'n verskeidenheid van geografiese gebiede versamel is het 'n paringstipe verhouding van 1:1 gehad. Dit is volgens verwagting in 'n bevolking wat vrylik paar. In spesifieke kwekerye was die geval egter anders. In die Suid-Afrikaanse kwekery was die *MAT-1* paringstipe oorheersend, terwyl *MAT-2* meer voorgekom het in Italië en V.S.A. Die hoë aantal hermafrodiete dui ook daarop dat die spesie onlangs ingebring is. DNA volgorde vergelykings was ook gebruik om variasie onder spesievlak te ondersoek. Alle isolate van Suid-Afrika, Australië, V.S.A. en 'n groep van Italië het identiese volgordes gehad. 'n Tweede groep in die Italiaanse bevolking is ook gevind met identiese DNA volgordes. In die Suid en Sentraal Amerikaanse bevolking is die meeste variasie gevind en sommige van die basis paar verskille is gedeel met *Cy. candelabrum*. Dit dui op 'n spesiasie in Suid Amerika.

In Deel 5 is die inligting wat verkry is vantevore uitgebrei na generiese vlak toe. Dit het 'n filogenie van alle bestaande *Calonectria* en *Cylindrocladium* spesies tot gevolg gehad, gebaseer op DNA volgordes van 'n deel van die β -tubulien geen. Verskeie klades is deur statistiese analise ondersteun. Verhoudinge tussen hierdie groepe was egter minder duidelik. Twee groot groepe was ook onderskei en die engste morfologiese karakter was met die geen filogenie ooreengestem het is die vorm van die "vesicle" op die kondiofore van die anamorf.

Tot slotsom, in Deel 6 is die verteenwoordigende groepe spesies van *Calonectria* en naby verwante genera vergelyk. Hierdie genera is alreeds voorheen bespreek onder die wye taksonomiese konsep *Nectria* sensu lato. 'n Geen boom gebaseer op β -tubulien was aangedui. Aan die hand van hierdie data en unieke anamorf verwantskappe is nuwe teleomorf genera voorgestel vir *Cylindrocladiella* (*Nectricladiella*), *Gliocladiopsis* (*Glionectria*) en *Xenocylindrocladium* (*Xenocalonectria*). Dit is ook bevind dat *Calonectria* monofileties is. Ag spesies van *Cylindrocladiella* is aangedui, waarvan twee teleomorfe het in *Nectricladiella*, naamlik *N. camelliae* (*Ce. microcylindrica*) en *N. infestans* (*Ce. infestans*).

Hierdie studie het dus bevind dat die huidige morfologies spesie konsepte in *Cylindrocladium* ook biologiese en filogeneties spesies omskryf. Die gebruik van paringstoetsers is aangedui as 'n goeie metode om morfologies eenderse spesies te onderskei. Dit wil egter voorkom asof filogenetiese spesie konspte gebaseer op DNA gebiede soos die β -tubulien en *MAT-2* geen, asook ander areas meer belangrik sal word vir verder taksonomies studies in hierdie swam.

"An acquaintance with fungi is in the highest degree necessary to man."

Linneaus C (1707-1778)

"Nothing in the whole world is coarse or despicable, but everything that the Divine Power has created and preserves is most worthy of contemplation...since in our judgement the very smallest of created things, equally as the greatest, have their miracles."

Holmskjold T (1732-1794)

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The Creator of us all.

"The classification of the Pyrenomycetes will never be either natural or philosophical, until the species become known in the most minute details of their frutification."

De Notaris G (1805-1877)

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1. An introduction to *Calonectria* and *Cylindrocladium* systematics

Background

The Hypocreales constitutes one of the 46 ascomycetous orders recognised in the current Dictionary of Fungi (Hawksworth et al 1995). Several members of this order have been studied extensively because they fill ecological niches that make them important in many fields of human endeavour. Hypocrealean species range from agents of biological control and producers of antibiotics to elicitors of potent mycotoxins (reviewed by Rossman 1996). The genus *Calonectria* De Not. is especially important and its members are pathogenic to a wide variety of plants in warm and humid conditions world-wide.

Descriptive accounts on the Hypocreales have centred primarily on species of the genera *Hypocrea* Fr., *Hypomyces* (Fr.) Tul. and *Nectria* (Fr.) Fr. (Rossman 1996). The genus *Nectria* sensu lato has been a repository for all species having fleshy, uniloculate ascocarps with a hypocrealean centrum with hyaline, non-apiculate, bicellular ascospores, and phialidic anamorphs (Rossman 1993). Recently several informal groupings of *Nectria* were re-described as genera within the family *Nectriaceae* (Rossman et al 1999). Genera were segregated from *Nectria* on the basis of single characters, including ascospore septation and conidiomatal morphology. *Calonectria*, relevant to this study, were already separately circumscribed previously by Rossman (1979b) and were one of these genera.

Teleomorph

The genus *Calonectria* (Ca.) was erected by De Notaris (1867) based on the type species *Ca. daldiniana* De Not. which has since proved to be a later synonym of *Ca. pyrochroa* (Desm.) Sacc. (Rossman 1979a). In Saccardo's description *Calonectria* was delimited for *Nectria*-like species with multiseptate ascospores (Saccardo 1883). Although Rossman (1979a, b, 1983) accepted differences in ascospore septation to exist at generic level, the concept of distinguishing genera on the basis of a single character was not supported. This viewpoint was in agreement with the work of Booth (1959) and subsequent authors who used a combination of characters that included anatomy of the perithecial wall, as well as ecology and presence of specific anamorphs for generic delimitation. Therefore, members of *Calonectria* were defined

as species with brightly coloured ascocarps that change colour when placed in 3% KOH solution (KOH+), have a warty to scaly wall structure, darkened stromatic base, and *Cylindrocladium* Morgan anamorphs (Rossman 1993, Rossman et al 1999).

A number of *Calonectria* species have been described in the previous two decades. Five species of *Calonectria* were accepted and monographed by Rossman (1983). A subsequent review included 10 species of *Calonectria* with *Cylindrocladium* anamorphs as well as an additional six *Cylindrocladium* species for which no *Calonectria* state was known (Peerally 1991). The most recent monograph was that by Crous and Wingfield (1994). These authors circumscribed sixteen *Calonectria* species with *Cylindrocladium* anamorphs, and seven *Cylindrocladium* species without known teleomorphs.

The emphasis of the last two monographs was firmly placed on features of the *Cylindrocladium* anamorph. The reasons for this are twofold. Firstly, the anamorph state is the form most frequently encountered in the field and secondly, nearly all species can be distinguished only on their asexual characters. This is due to the fact that ascospore size, as well as ascus and perithecial morphology can only place taxa into one of three species complexes. Furthermore, in many cases, species are heterothallic, and the *Calonectria* state is rarely observed. In line with the arguments presented above, preference in this document will be given to the terminology and morphological characters of the *Cylindrocladium* state in the rest of this study.

Anamorph

The asexual genus, *Cylindrocladium* (Cy.), was first erected by Morgan (1892) for a species found growing on an old pod of *Gleditsia triacanthos* L. in Ohio, U.S.A. This genus was delimited by the author as follows "Sterile hyphae, creeping, branched forked or trichotomously branched the sporophores in pairs or threes at the extremities of the branchlets and cymosely arranged; spores solitary, cylindrical, 1-septate, hyaline". The type species was described as *Cy. scoparium* Morgan. Most notably, no mention was made of the appendage on the conidiophores. However, subsequent descriptions by Massey (1917) and Anderson (1918) clearly indicated this feature, which later authors found to be so characteristic for *Cylindrocladium* (Boedijn & Reitsma 1950, Sobers & Seymour 1967, Peerally 1991, Crous & Wingfield 1994). Presently four conidial types are known for *Cylindrocladium*, namely chlamydospores, microconidia, macroconidia and megaconidia (Crous & Seifert 1998). *Cy. scoparium*, however, forms macroconidia and chlamydospores only. As

no cultures were obtained by Morgan (1892), nor any mention made of chlamydospores, it is clear that the generic name represents the macroconidial form.

Several authors described species under different generic names that were eventually synonymised under *Cylindrocladium*. *Diplocladium cylindrosporum* Ellis & Everh. was described with a sterile appendage, swollen at the tip, but was subsequently redispersed to *Cylindrocladium* by Boedijn and Reitsma (1950). A similar fate was in store for the genera, *Candelospora* Hawley apud Rea & Hawley (Boedijn & Reitsma 1950), *Tetracytium* Vanderwalle (Subramanian 1971) and *Cylindrocladiopsis* J.M. Yen (Crous & Seifert 1998).

Species of *Cylindrocladium* have been defined based on various features of the conidiophores and the conidia (Crous & Wingfield 1994). The use of one such character, the stipe emanating from the conidiophores and the shape of its apical vesicle has elicited much difference of opinion. Before the important review done by Boedijn and Reitsma (1950), taxonomists concentrated mainly on conidial morphology. The importance of the presence of a vesicle and its shape as a species defining character was only emphasised several years later (Bell & Sobers 1966, Sobers & Seymour 1967). However, subsequent authors rejected this species concept due to the variability of this feature (Hunter & Barnett 1978, Rossman 1983). Peerally (1991) argued that vesicle shape can be a reliable taxonomic character in fresh cultures and that this must be used in combination with conidial characters for identification of *Cylindrocladium* species. This view was also supported by subsequent authors (Crous et al 1992, Uchida & Aragaki 1992b). Crous et al (1992) showed that the osmotic potential of the medium influences vesicle shape and that vesicle morphology can be a reliable character when standardised media and growth conditions are applied. Consequently, this approach was combined with other morphological characters in order to delimit several *Cylindrocladium* species (Crous & Wingfield 1994).

Closely related genera

The anamorph genus *Cylindrocladiella* Boesew. was erected by Boesewinkel (1982) to accommodate several small-spored species that were previously placed in *Cylindrocladium*. *Cylindrocladiella* (Ce.) was reported to have different conidiophore branching patterns, conidial shapes, dimensions, as well as cultural characteristics. The recognition of *Nectria camelliae* Shipton as the teleomorph for one of these species made a strong case for the separation of *Cylindrocladiella*. More recent

studies have confirmed the genera *Cylindrocladium* and *Cylindrocladiella* to be distinct (Crous & Wingfield 1993, Crous et al 1994, Victor et al 1998). Samuels (1991) allocated *N. camelliae* (anamorph: *Ce. infestans*) to *Nectria* subg. *Dialonectria*, while (Rossman et al 1999), in a re-evaluation of the group, placed it in *Cosmospora* (Cs.) as *Cs. camelliae* (Shipton) Rossman & Samuels, based on its teleomorph morphology. In comparison to *Calonectria*, the perithecial walls of *Cs. camelliae* are smooth and narrow, while the ascospores are 1-septate and much smaller.

Several genera with characters morphologically similar to those of *Calonectria* have previously been described under the generic concept of *Nectria* sensu lato in the *Nectriaceae* (Rossman et al 1999). Molecular character based phylogenies in this group have largely confirmed morphological groupings. Sequence comparisons of the nuclear large-subunit (28S) ribosomal DNA obtained from several genera in the Hypocreales indicated that some clustered closely to *Calonectria* (Rehner & Samuels 1994). *Leuconectria clusiae* Rossman et al (anamorph: *Gliocephalotrichum bulbilium* J.J. Ellis & Hesselst.), as well as *Nectria radiculicola* Gerlach & Nilsson (anamorph: *Cylindrocarpon destructans* (Zinnsm.) Scholten) showed the closest similarity, with two typical species of *Nectria*, *N. pseudotrichia* Berk. & Kurt. [anamorph: *Tubercularia lateritia* (Berk.) Seifert] and *N. cinnabarina*, forming part of this subclade, but grouping more distantly.

In morphological studies, several similarities were found between the *Gliocephalotrichum* and *Cylindrocladium* anamorphs of *Leuconectria* and *Calonectria* (Rossman & Samuels 1993). The most notable was the formation of cylindrical conidia, penicillate conidiophores, and a brown pigment diffusing in agar media. Perithecial anatomy in *N. radiculicola* and its relatives was also observed to be similar to that of *Calonectria* (Samuels & Brayford 1990). Samuels and Seifert (1987) also recognised similarity between *Cylindrocladium* and the *Cylindrocarpon* Wollenw. (Co.) anamorphs of *N. radiculicola*.

In addition to these genera, several other anamorph form genera are similar to *Cylindrocladium*, having cylindrical macroconidia and phialidic conidiogenous cells. Among these are *Gliocladiopsis* S.B. Saksena, *Xenocylindrocladium* Decock et al and *Curvocladium* Decock & Crous. Of these genera, only *Xenocylindrocladium* (Decock et al 1997) has been linked to a teleomorph, forming part of the *Nectria* sensu lato clade.

Characterisation of *Cylindrocladium* and *Calonectria* species

Morphology and cultural characteristics

As mentioned previously, the most recent taxonomic concept for *Calonectria* places emphasis on the features of its *Cylindrocladium* anamorph. The standardisation of growth conditions (Peerally 1991, Crous et al 1992, Crous & Wingfield 1994) enabled the use of characters previously described as variable and unreliable (Hunter & Barnett 1978, Rossman 1983). Besides the shape and size of the apical vesicles, species are differentiated on the dimensions and septation of conidia, phialide shape, stipe length, conidiophore branching pattern and cultural characteristics. Teleomorph characteristics evaluated for interspecies differentiation include ascospore size and septation, perithecial colour and morphology.

In addition to these characters, the taxonomic value of the occurrence of micro- and megaconidial states has also been evaluated. Sobers (1968) discussed the presence of a small-spored form of *Cy. pteridis* Wolf that was observed in cultures growing on water agar and on plant material. This microconidial form has also been reported in at least eight of the *Cylindrocladium* species treated in the monograph of Crous and Wingfield (1994). Microconidia are generally cylindrical, straight or curved and 1-septate, although 3-septate conidia have been reported for *Cy. multiseptatum* and *Cy. rumohrae* (El-Gholl et al 1997, Crous et al 1998b). Crous and Wingfield (1994) questioned the usefulness of this character for taxonomic studies however, as the microconidia are not produced by all strains of a species.

The term “megaconidia” was only recently defined as a fourth conidial type for *Cylindrocladium* (Crous & Seifert 1998). This conidial state has been infrequently reported before (Sobers 1971, Alfieri et al 1972, Uchida & Aragaki 1992a). In agreement with the terminology used for *Fusarium* conidia, “normal” conidia are referred to as macroconidia, while the larger conidial type has been termed as megaconidia (Crous & Seifert 1998). Megaconidia were reported for four species of *Cylindrocladium* and were described as multiseptate, widest in the middle, straight to curved or bent at right angles, and significantly larger than macroconidia. As in the case of microconidia the value of this as a taxonomic character is limited, but can be important in cases where some strains form only these conidia (Crous & Seifert 1998).

The functions and roles of the mega- and microconidial states in the *Calonectria* life cycle are still uncertain and open to speculation. The occurrence of microconidial states is not unique in the *Nectriaceae* and it is regularly found in *Cylindrocarpon* and *Fusarium* Link:Fr. species (Booth 1971). Another conidial state intermediate between micro- and macroconidia was termed mesoconidia for species of *Fusarium* (Pascoe 1990a). Mesoconidia are thought to be produced under dry conditions in order to allow air dispersal (Pascoe 1990b). A similar relationship with regard to specific environmental conditions and functions of micro- and megaconidia may occur in *Cylindrocladium*.

Cardinal temperature requirements for growth, as well as the production of chlamydospores and microsclerotia were evaluated by Crous & Wingfield (1994). Several species were found to grow at either high or low temperatures, and to produce sparse or extensive amounts of chlamydospores on malt extract agar. Although chlamydospore formation influences colony colour, these characters were found to be of much less taxonomic value than in related hypocrealean genera (Crous & Wingfield 1994).

Physiological and biochemical characteristics

The response of a number of *Cylindrocladium* species to various nutritional and environmental conditions has been studied by Hunter and Barnett (1978). No major variations were observed in utilisation of different C and N sources, although species differed in sporulation. However, different C sources had an effect on microsclerotial production (Weaver 1974), while the ratio of C to N also influenced this character (Hunter & Barnett 1975). Long term storage and excessive subculturing resulted in sterility in older cultures, which could only be observed as white mycelium (Hunter & Barnett 1978). Variations were found in thiamine sufficiency, and effects of light on sporulation. Optimum temperatures for growth were found to vary between 25-30°C for all species studied (Hunter & Barnett 1978). Other biochemical studies included aminopeptidase substrate specificities used by Stevens et al (1990) to distinguish *Cylindrocladium* pathogens found in Wisconsin, U.S.A. Recent work was also done to determine the structures of acidic fungal polysaccharides isolated from cell-walls of *Cylindrocladium* species by means of ¹³C NMR spectroscopy (Ahrazem et al 1997). This study revealed the usefulness of using these markers for chemotaxonomy and emphasised the possibilities of finding new polysaccharidic structures in fungal cell walls.

Molecular characteristics

Protein characterisation

Because of the similarity and variability in several morphological characters used for *Calonectria* and *Cylindrocladium* taxonomy, the use of molecular characters has become increasingly important. Several molecular characters have been applied in attempts to solve problems relating to phylogeny and the identification of species. Total proteins and isozyme analysis have been used extensively to distinguish species in numerous fungal genera (Alfenas 1998). In *Cylindrocladium* taxonomy, total protein and isozyme profiles have been used to aid in the delimitation of species (Crous et al 1993a, b, c, El-Gholl et al 1993, El-Gholl et al 1997), and to investigate variation below species level (Crous et al 1998a). However, environmental conditions can influence protein expression and thus invalidate some results (Michelmore & Hulbert 1987).

DNA characterisation

Restriction fragment length polymorphisms (RFLPs) have been used for several years for fungal population studies and taxonomy for several years (McDonald & McDermott 1993). In *Cylindrocladium* RFLPs from nuclear DNA has been applied, together with morphological observations to support proposals for several new species (Crous et al 1995, Crous et al 1997a, Crous et al 1997b) and has also indicated variation within existing species (Overmeyer et al 1996, Jeng et al 1997). Based on these data, some species were shown to be conspecific (Crous et al 1995). Other DNA based molecular characters were obtained through random amplified polymorphisms (RAPDs) (Overmeyer et al 1996, El-Gholl et al 1997, Victor et al 1997) and AT-DNA profiles (Victor et al 1997).

DNA sequence comparisons are being used increasingly frequently in fungal systematics. In the Hypocreales, numerous phylogenies using DNA sequence comparisons from a wide variety of loci have already been made at several levels (e.g. O'Donnell 1993, Spatafora & Blackwell 1993, Rehner & Samuels 1994, Rehner & Samuels 1995, Glenn et al 1996, O'Donnell et al 1998). In most cases this has led to a better understanding of the underlying morphological phylogeny. The first sequence data for *Calonectria* spp. were obtained by O'Donnell (1993) and subsequently by Rehner and Samuels (1994). In this study (O'Donnell 1993) the DNA sequence of the 5' end of the 28S ribosomal RNA gene from an isolate identified as *Ca. pyrochroa* was included in a comparison of various other hypocrealean species with *Fusarium* anamorphs. The second study (Rehner &

Samuels 1995) compared a wider array of hypocrealean species. Subsequent sequencing data was obtained from the 5.8S ribosomal RNA gene and the two flanking internally transcribed spacers (ITS1 and ITS2) of several isolates by Hamelin (1996) in order to devise primers for detection of *Cy. floridanum* Sobers & C. P. Seym. and *Co. destructans* in nursery seedlings. DNA sequence comparisons between *Cylindrocladium* species were made by Jeng et al (1996) when isolates of *Cy. floridanum* were compared with *Cy. scoparium* using the DNA sequences obtained from the same genomic area. Although the authors did not do a phylogenetic analysis, differences could be ascertained between these two species. Besides larger molecular based studies done on hypocrealean and other species that included 28S rRNA sequences from *Cy. floridanum* and *Cy. scoparium* (O'Donnell 1993, Rehner & Samuels 1995, Ogawa et al 1997), no DNA sequence based phylogeny of *Cylindrocladium* at species and generic level has yet been published.

***Calonectria* and *Cylindrocladium* as plant pathogens**

Since the first description of *Cylindrocladium scoparium* (Morgan 1892) was made from material collected on dead pods of honey locust, it created the impression that this species may be saprophytic. However, the first reports of a plant disease caused by this fungus were by Massey (1917) and subsequently by Anderson (1918). These authors described the fungus as the causal agent of crown cankers on roses. Since then *Cy. scoparium* has been associated with a wide range of disease problems in over 30 plant families throughout the world (Booth & Gibson 1973, French & Menge 1978, Peerally 1991, Waipara et al 1996). This is also true for other species in *Cylindrocladium* (e.g. Bell & Sobers 1966, Cordell & Rowan 1975, French & Menge 1978, Mohanan & Sharma 1985, Chase & Poole 1988, Peerally 1991, Koike et al 1999). Prominent diseases caused by *Cylindrocladium* spp. include *Cylindrocladium* black rot (CBR), a devastating pod and root necrosis disease of peanuts caused by *Cy. parasitica* Crous et al (Porter et al 1984), *Cylindrocladium* cutting rot of *Eucalyptus* caused by several *Cylindrocladium* spp. (Ferreira 1989) and *Cylindrocladium* root and petiole rot of *Spathiphyllum* by *Cy. spathiphylli* (Chase & Poole 1988), to name but a few. Symptoms caused by other *Cylindrocladium* spp. include damping-off, root rot, crown canker, leaf spot, seedling and shoot blight, needle blight, wilt, fruit rot, tuber rot, cutting rot, die-back and stem lesions.

Samuels 1995) compared a wider array of hypocrealean species. Subsequent sequencing data was obtained from the 5.8S ribosomal RNA gene and the two flanking internally transcribed spacers (ITS1 and ITS2) of several isolates by Hamelin (1996) in order to devise primers for detection of *Cy. floridanum* Sobers & C. P. Seym. and *Co. destructans* in nursery seedlings. DNA sequence comparisons between *Cylindrocladium* species were made by Jeng et al (1996) when isolates of *Cy. floridanum* were compared with *Cy. scoparium* using the DNA sequences obtained from the same genomic area. Although the authors did not do a phylogenetic analysis, differences could be ascertained between these two species. Besides larger molecular based studies done on hypocrealean and other species that included 28S rRNA sequences from *Cy. floridanum* and *Cy. scoparium* (O'Donnell 1993, Rehner & Samuels 1995, Ogawa et al 1997), no DNA sequence based phylogeny of *Cylindrocladium* at species and generic level has yet been published.

***Calonectria* and *Cylindrocladium* as plant pathogens**

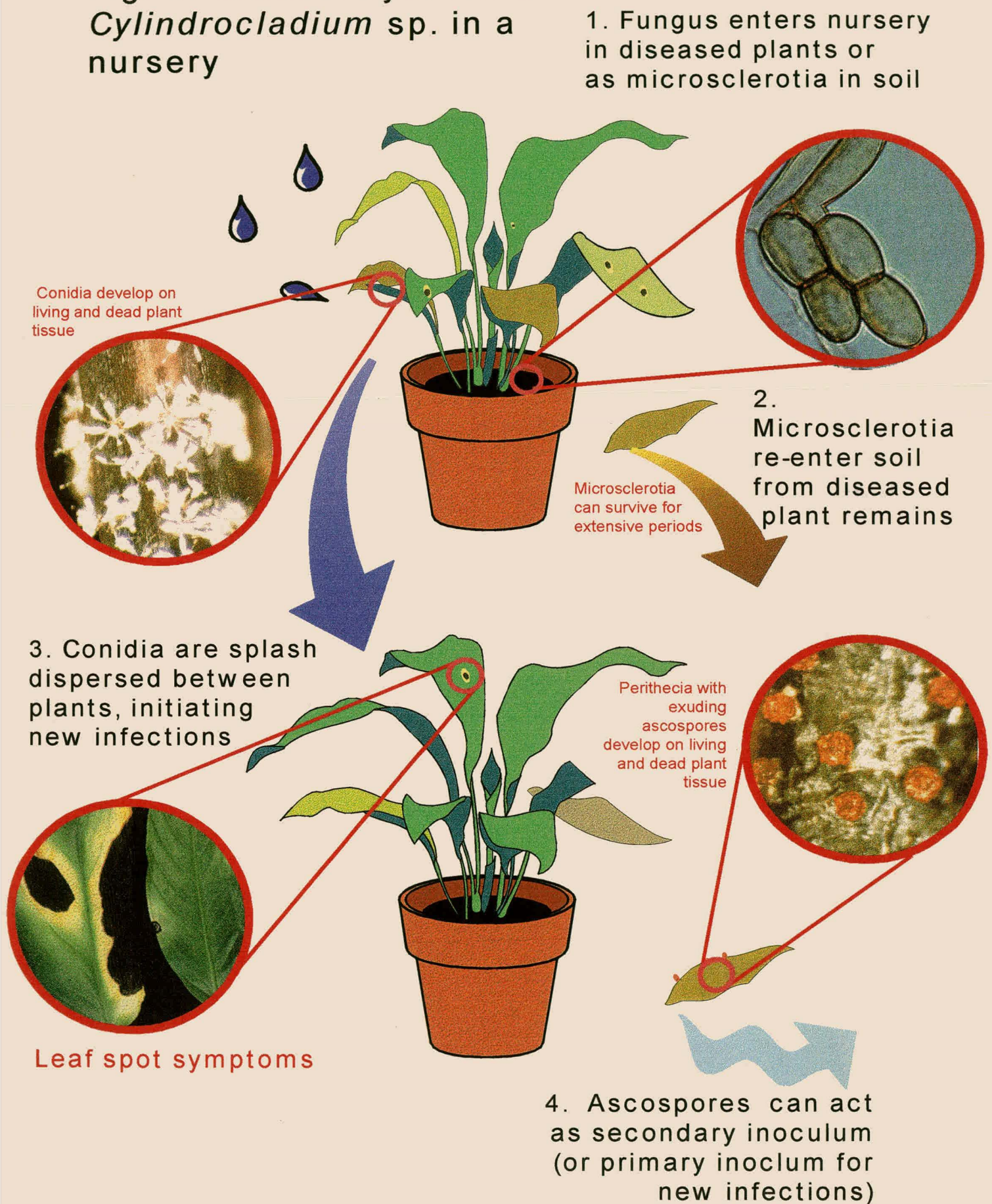
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Research on the pathology of *Cylindrocladium* and *Calonectria* species has concentrated mainly on the identification of the pathogens, tests for pathogenicity, the role of microsclerotia in disease aetiology and control through chemical means (Peerally 1991). In addition to this, several comparative studies have shown differences between pathogenicity and *in vitro* fungicide resistance of various species (Sobers & Litrell 1974, Sharma & Mohanan 1991a, Blum et al 1992, Sharma & Mohanan 1992). Less is known of variation below species level. Studies by Rowe and Beute (1975) done on the pathogenicity of various *Cy. parasitica* (as *Cy. crotalariae*) isolates showed no variation for isolates from different geographic origins, although recent observations (B. Shrew, pers. comm.) suggest that such variation may well occur in the U.S.A. Furthermore, results by Sharma (1991b) provided evidence that physiological strains exist in *Cy. quinqueseptatum*.

The infection process of *Cylindrocladium* spp. appears to be similar on a range of hosts. This is summarised in Fig. 1. Usually infection of plants in nurseries and plantations comes from diseased plant material or soil originating from adjacently infected areas, or by transportation (Anderson 1918, Thies & Patton 1970, Crous et al 1991). The primary propagule for nursery infections has been determined to be microsclerotia, consisting of chains or clusters of chlamydospores (Thies & Patton 1970). Additional infection of plants within nurseries and plantations can occur through splash dispersal of conidia (Mohanani & Sharma 1986), or wind-born ascospores. Perithecia can also develop on infected material and act as a source of inoculum (Crous et al 1991). To date no research has been conducted at the population level to establish what role the sexual and asexual propagules play in establishing genetic variation in the disease life cycle of *Calonectria*.

Usually the presence of free water is essential for germination of the infectious propagule to occur (Anderson 1918, Anderson et al 1962). Colonisation of plant leaves and stems has been observed after inoculation with conidia and appressorium formation occurs 4 h after inoculation for *Cy. quinqueseptatum* (Sharma & Mohanan 1990). Chlamydospores and microsclerotia were described developing in several plant tissues (Anderson 1918, Bugbee & Anderson 1963). This infected plant material can release the microsclerotia into the soil when infected plant remains fall on the ground where they can survive without a host for periods of up to 15 years or more (Sobers & Litrell 1974, Crous et al 1991).

Fig. 1. Disease cycle of a *Cylindrocladium* sp. in a nursery



Conclusions

Although the phylogenetic placement of *Calonectria* within the Hypocreales has been studied previously, the interspecies phylogeny of *Calonectria* has only been determined through morphological comparisons and molecular markers such as RFLPs and RAPDs. Sequence determinations have been made, but no DNA based phylogenetic study has yet been carried out on species in this genus. Because *Calonectria* species are common as the causal organisms of economically important plant diseases (mainly as *Cylindrocladium* spp.) world-wide, accurate identification of different species is essential. A phylogenetic assessment of the various species in the genus would therefore aid species identification. It would also facilitate a re-evaluation of the significance of the various morphological characters previously used for species identification. This information would aid studies into the diversification and mating isolation of species in *Calonectria*.

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2. The *Cylindrocladium candelabrum* species complex includes four distinct mating populations*

Abstract

Cylindrocladium candelabrum-like isolates were collected from a wide variety of geographic locations and compared based on their morphology, sexual compatibility and the nucleotide sequences of their rDNA ITS regions. All isolates included in this study mated to produce *Calonectria* teleomorphs with viable progeny. Four distinct mating populations were identified, each representing a genetically isolated, biallelic, heterothallic population. Several representative isolates of each mating population, reflecting geographic diversity, were chosen for sequence comparisons. The internal transcribed spacer (ITS) regions 1 and 2 that flank the 5.8S rDNA gene, as well as the gene itself, were sequenced and compared. All isolates representing the same group yielded similar sequences, but small, consistent differences were found between the groups. Based on these results we recognise *Calonectria scoparia* (anamorph *Cylindrocladium candelabrum*), and describe three new species, namely *Calonectria pauciramosa* (anamorph *Cylindrocladium pauciramosum*), *Calonectria insularis* (anamorph *Cylindrocladium insulare*) and *Calonectria mexicana* (anamorph *Cylindrocladium mexicanum*).

Introduction

Cylindrocladium scoparium Morgan, the type species of *Cylindrocladium* Morgan (Cy.) (Morgan 1892), has been associated with a wide range of plant disease problems in over 30 families throughout the world (Booth & Gibson 1973, French & Menge 1978, Peerally 1991, Waipara et al 1996). This species is, however, the most commonly incorrectly identified taxon in the genus. *Cy. scoparium sensu stricto* has been confirmed from only North America, but has possibly also been introduced into Europe (Overmeyer et al 1996).

Cylindrocladium scoparium, still incorrectly treated by many researchers as synonymous with *Cy. floridanum* Sobers & C. P. Seym., has been the subject of

* Published: Schoch CL, Crous PW, Wingfield BD, Wingfield MJ. 1999. The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* 91: 286-298.

much controversy. Victor et al (1997) used morphology, sexual compatibility, RAPD markers and A+T-rich total DNA polymorphisms to compare *Cy. scoparium* (teleomorph *Calonectria morganii* Crous et al), *Cy. candelabrum* Viégas (teleomorph *Ca. scoparia* Peerally), *Cy. ovatum* El-Gholl et al (teleomorph *Ca. ovata* D. Victor & Crous) and *Cy. floridanum* (teleomorph *Ca. kyotensis* Terash.). This study showed that these species represent distinct taxa. Furthermore, evidence was presented to show that more than one species possibly exists in the *Cy. floridanum* complex. Additionally, based on DNA fingerprinting with human minisatellite DNA as a probe, Jeng et al (1997) showed the presence of three groups of isolates in collections of *Cy. floridanum* from Canada and the U.S.A.

Among the small-spored species of *Cylindrocladium*, *Cy. scoparium* has also commonly been confused with taxa such as *Cy. ovatum* and *Cy. candelabrum*. All three of the latter species are heterothallic. In a recent study Crous et al (1998) confirmed the biallelic, heterothallic nature of *Cy. ovatum*. In earlier studies, however, very low mating percentages were obtained for *Cy. candelabrum* and *Cy. scoparium* (Crous et al 1993a, Overmeyer et al 1996), suggesting that further research was required to elucidate their mating systems.

Cylindrocladium candelabrum, which was originally described from leaves of a *Luma* sp. in Brazil, was characterised by Viégas (1946) as having narrowly ellipsoidal vesicles and 1-septate conidia, 40-88 x 5-6 µm. Crous et al (1993a) re-examined the type specimen (IACM 440), and found it to be almost completely devoid of material, but the few conidia that were observed were 46-70 x 3.5-5 µm, and the vesicles were ellipsoidal to narrowly obpyriform. A neotype (PREM 51045) was subsequently designated, and two isolates PPRI 4153 and 4163 identified as the two mating tester strains.

The species concept of *Cy. candelabrum* was complicated by Peerally (1991) who considered it synonymous with *Cy. ellipticum* Alfieri et al. The latter species was later shown to be a synonym of *Cy. scoparium* (Crous et al 1993a). To readily distinguish these species, *Cy. scoparium* was circumscribed as having ellipsoidal to pyriform vesicles (widest above the middle), while those of *Cy. candelabrum* were ellipsoidal to obpyriform (widest below the middle). However, a high degree of plasticity was observed amongst *Cy. candelabrum*-like isolates. This was particularly true in their vesicle shape, conidiophore branching pattern and conidial dimensions. Due to the low mating type frequency of isolates in previous studies, no clear

indication was obtained on the nature and relevance of this variation amongst *Cy. candelabrum* isolates, and the species was accepted as being highly variable.

Molecular tools have become increasingly useful in providing additional evidence that has supported the interpretation of morphological variation. Several techniques including protein profiles (Crous et al 1993a), RAPDs (Victor et al 1997) and RFLPs (Crous et al 1997b), have been applied to the taxonomy of *Cylindrocladium* spp. The nucleotide sequences of the ribosomal DNA (rDNA) region contain intermittent functional and non-functional regions (Furlong et al 1983). The more conserved rDNA genes allow for comparisons between higher taxa. For example, Rehner and Samuels (1995) compared the nucleotide sequences of the 28S rDNA gene from a wide range of hypocrealean taxa, including *Cy. scoparium* and *Cy. floridanum*. More variable areas are provided by intergenic regions such as the internal transcribed spacers (ITS1 and ITS2) that flank the 5.8S rDNA gene. Various researchers have used these sequences to resolve intra- and interspecies phylogenies (Nazar et al 1991, Sreenivasprasad et al 1994, Bryan et al 1995, Jeng et al 1996, Witthuhn et al 1998).

Recently Jeng et al (1997) published ITS1, ITS2 and 5.8S rDNA sequences of *Cy. scoparium* and *Cy. floridanum*. In these comparisons, one six base pair nucleotide deletion and three point mutations were found in the ITS2 region. This indicated the potential of this region to be used as a tool to differentiate between morphologically similar *Cy. candelabrum*-like species. Accordingly, the present study was undertaken to investigate the application of a biological species concept as well as a phylogenetic species concept to isolates provisionally accommodated in the *Cy. candelabrum* species complex. Using these data, it was possible to evaluate the value of morphological characters in *Cylindrocladium*.

Materials and Methods

Isolates

Cylindrocladium candelabrum isolates were either obtained from symptomatic material, or they were baited from soil samples. Soil samples were collected and treated as explained in Crous et al (1997a). Type specimens were lodged at the National Collection of Fungi in Pretoria (PREM), and ex-type cultures maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U).

Table I. Isolates selected for sequencing.

Species	STE-U no.	Origin
<i>Cy. pauciramosum</i> (Group 1)	951	Mexico
	971	South Africa
	1160	Colombia
	1691	Australia
<i>Cy. candelabrum</i> (Group 2)	1674	Brazil
	1675	Brazil
	1676	Brazil
	1678	Brazil
<i>Cy. insulare</i> (Group 3)	766	Madagascar
	768	Madagascar
	616	Brazil
	954	Mexico
<i>Cy. mexicanum</i> (Group 4)	927	Mexico
	928	Mexico
	941	Mexico
	966	Mexico

Sexual compatibility

One hundred single conidial *Cy. candelabrum*-like isolates (listed under the results), originating from various geographic locations were mated in all possible combinations. This was achieved by removing 3 mm diam agar plugs from the periphery of actively growing cultures and placing them on CLA plates as described by Crous et al (1993a). Two different isolates were placed in a Petri dish with carnation leaves between them. Following this, plates were packed in stacks of 10, sealed in plastic bags and incubated on the laboratory bench at 22°C. Protoperithecia appeared after 2 wk and successful matings were determined after 2 mo of incubation. Successful matings were regarded as those isolate combinations that produced perithecia with fertile, extruding ascospores.

Mating groups were subsequently distinguished and strains that resulted in prolific matings were selected from each group. For each mating group identified, ascospores were obtained from two matings, involving four separate isolates. Seven single ascospores were sub-cultured for each mating group, and these were crossed in all possible combinations in order to reconfirm the biallelic, heterothallic nature of each mating population. Two isolates of opposing mating type were selected as tester strains from these isolates, and these were subsequently mated with the tester strains of the other groups to reconfirm that no mating was occurring between groups.

Both strands of the ITS1 and ITS2 intergenic spacers as well as the 5.8S ribosomal gene were sequenced and compared. Sequences were deposited at GenBank (AF059280-AF059283). DNA was amplified using the primers ITS1 (5'-dTCCGTAGGTGAACCTGCGG) and ITS4 (5'-dTCCTCCGCTTATTGATATGC) (White et al 1990). The region amplified was the 5.8S ribosomal gene and the two internal transcribed spacers (ITS1 and ITS2) flanking the gene. PCR amplifications were performed on a Hybaid Omnigene Temperature Cycler (Hybaid, Middlesex, U.K.). Reactions comprised of 1 µl Expand High Fidelity DNA polymerase (Boehringer Mannheim, Mannheim, Germany) and 1 µl reaction buffer containing 1.5 mM MgCl₂ (Boehringer Mannheim), with MgCl₂ added to make up the final buffer concentration to 5.5 mM. Liquid paraffin oil was overlaid to prevent evaporation. Other reagents added to the final volume of 100 µl were 250 µM of each NTP, 0.5 µM of each primer and 25 ng DNA. PCR conditions were a denaturing step at 94°C for 1 min followed by 10 cycles of 56°C for 30 s, 72°C for 2 min and 94°C for 15 s. This was followed by a further 20 cycles at the same settings except for a 20 s time increase at 72°C.

PCR products were purified using Wizard PCR Preps (Promega Corporation, Madison, Wisconsin). Both strands of the PCR product were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). A Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase (Perkin-Elmer) was used for the sequencing reactions. The reactions were carried out with a concentration of 20 to 40 ng of DNA template and 3.2 pmol primer in a total volume of 10 µl. The cycle sequencing reaction was done by PCR under conditions of 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. This was repeated for 25 cycles. DNA was finally purified using Centri-Sep Spin columns (Princeton Separations, Adelphia, New Jersey) and loaded onto the sequencing gel.

Phylogenetic analysis of the ITS1 and ITS2 DNA sequences was performed by using the PAUP (Phylogenetic Analysis Using Parsimony) 3.1.1 program (Swofford 1993). The branch and bound algorithm, with gaps treated as a fifth character was used. Confidence intervals were determined using a 1000 bootstrap replications. All uninformative characters were ignored. Sequences of *Cy. scoparium* and *Cy. floridanum*, previously published by Jeng et al (1997), were used for comparison. In addition to this, a sequence of *Fusarium subglutinans*, deposited by Waalwijk et al (1996), was obtained (EMBL accession number X94167) and used as outgroup.

Morphological comparisons

Isolates were cultured on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa), plated onto carnation-leaf agar (CLA) (Crous et al 1992), incubated at 25°C under near-ultraviolet light, and examined after 7 d. Only material occurring on carnation leaves was examined. Mounts were prepared in lactophenol, examined under Nomarski and phase contrast, and measurements made at x 1000 magnification. Wherever possible, each measurement represents at least 30 observations, and extremes are given in parentheses. Cardinal temperature requirements for growth and cultural characteristics were determined after 6 d on MEA, using procedures described by Crous and Wingfield (1994), and colony colours coded according to Rayner (1970). Cultures of *Cy. candelabrum* were identified using the keys of Crous and Wingfield (1994).

Results

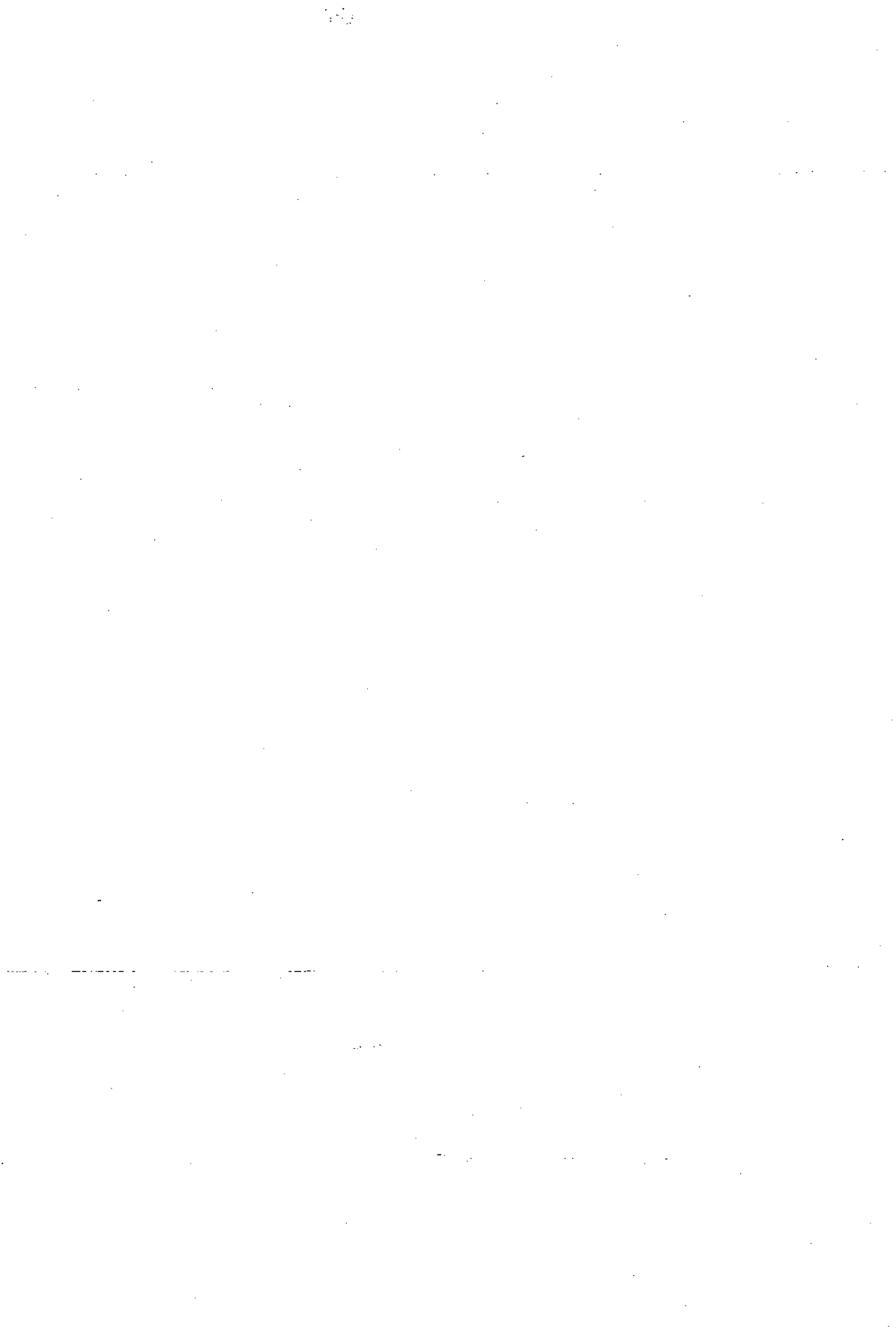
Sexual compatibility

All matings between the selected isolates resulted in perithecia containing fertile ascospores, except where STE-U 216 was concerned (Fig. 1). Whether this isolate constitutes another mating population, or has lost the ability to mate, remains unresolved. Control inoculations indicated that all isolates used were self sterile. Isolates of the same mating type yielded no perithecia when mated, confirming the biallelic, heterothallic mating system commonly found in ascomycetes (Yoder et al 1986). Four distinct mating populations (Groups 1-4) were observed. No successful matings were observed between the different mating groups, and subsequent crossings between ascospore progeny of prolific mating strains confirmed the distinctiveness of the mating groups (results not shown).

Sequence analysis

No differences were detected between isolates for their 5.8S sequences. The four isolates selected per mating group (Table 1), revealed ITS sequences that were 100 % similar within each group, irrespective of geographic location. For the purpose of comparison a single sequence, representing the four isolates from one species, was subsequently used to compare isolates of the four mating populations. A number of single and double base pair substitutions and deletions were found between all the species in the ITS1 and ITS2 regions (Fig. 2).

Fig1



	60
<i>Cy. floridanum</i>	CCGAGTTTACAACCTCCCAACCCCATGTGAACATACCTGTTTCGTTCCCTCGGCGGTGTC
<i>Cy. scoparium</i>	-----
<i>Cy. pauciramosum</i>	-----
<i>Cy. candelabrum</i>	-----
<i>Cy. insulare</i>	-----
<i>Cy. mexicanum</i>	-----
	120
<i>Cy. floridanum</i>	CGGCAACGGCCCGCCAGAGGACCCAACTCTTTTGAATTTTCAGTATCTTCTGAGT
<i>Cy. scoparium</i>	-----
<i>Cy. pauciramosum</i>	-----
<i>Cy. candelabrum</i>	-----
<i>Cy. insulare</i>	-----
<i>Cy. mexicanum</i>	-----
	180
<i>Cy. floridanum</i>	AAAAAAACAA*TAAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAA
<i>Cy. scoparium</i>	-----*
<i>Cy. pauciramosum</i>	-----**--A
<i>Cy. candelabrum</i>	-----**--A
<i>Cy. insulare</i>	-----*
<i>Cy. mexicanum</i>	G-----*
	240
<i>Cy. floridanum</i>	<u>GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT</u>
<i>Cy. scoparium</i>	-----
<i>Cy. pauciramosum</i>	-----
<i>Cy. candelabrum</i>	-----
<i>Cy. insulare</i>	-----
<i>Cy. mexicanum</i>	-----
	300
<i>Cy. floridanum</i>	<u>TGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAA</u>
<i>Cy. scoparium</i>	-----
<i>Cy. pauciramosum</i>	-----
<i>Cy. candelabrum</i>	-----
<i>Cy. insulare</i>	-----
<i>Cy. mexicanum</i>	-----
	360
<i>Cy. floridanum</i>	CCCTCAAGCACTTCGGGAGCTTGGTGTGTTGGGGATCGGCAGGGCGTC*TCCGGGTCCGCGCC
<i>Cy. scoparium</i>	-----T-*****-A-----*
<i>Cy. pauciramosum</i>	-----T-A*****-A-----C-----
<i>Cy. candelabrum</i>	-----T-A*****-A-----G-C-----
<i>Cy. insulare</i>	-----T-*****-A-----C-----
<i>Cy. mexicanum</i>	-----T-A*****-A-----C-----
	400
<i>Cy. floridanum</i>	GTCCCCCAAATCTAGTGGCGGTCTCGCTGTAGCTTCCTCTGCGTAGTAATACACCTCGCT
<i>Cy. scoparium</i>	-----A-----
<i>Cy. pauciramosum</i>	-----T-----
<i>Cy. candelabrum</i>	-----A-----
<i>Cy. insulare</i>	-----A-----
<i>Cy. mexicanum</i>	-----T-----
	448
<i>Cy. floridanum</i>	CTGGAGTCTCGGTGCG*CCACGCCGTAAAACCCCAACTTTTTTCTGG
<i>Cy. scoparium</i>	-----*-----*
<i>Cy. pauciramosum</i>	-----G-----*
<i>Cy. candelabrum</i>	-----A-----*
<i>Cy. insulare</i>	-----G-----T-----
<i>Cy. mexicanum</i>	-----G-----*

Fig. 2. Nucleotide comparison of the rDNA ITS region of *Cylindrocladium* isolates. *Cylindrocladium scoparium* and *Cy. floridanum* are included for comparison with the consensus sequences of each mating population (biological species) shown as indicated. The sequence of *Cy. floridanum* is shown in full. Asterisks indicate sites of nucleotide deletion. Sequences are shown beginning with the 5' end of ITS1, followed by the 5.8 S gene shown underlined and the 3' end of ITS2.

Previous work done by Jeng et al (1997) showed one six base pair deletion and 3 single base substitutions when the sequences of *Cy. floridanum* and *Cy. scoparium* were compared. None of the other species sequenced contained a six base pair deletion found in the *Cy. floridanum* ITS2 region.

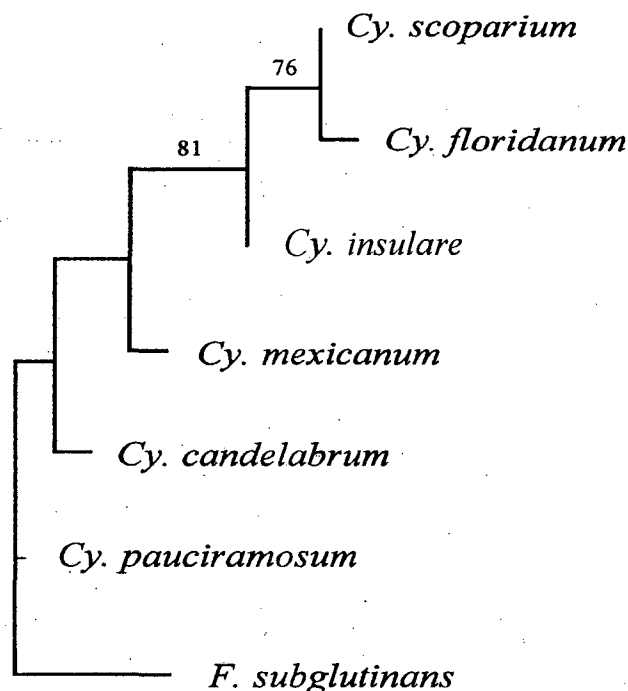


Fig. 3. Phylogeny of the species in the *Cylindrocladium candelabrum* complex. One of four most parsimonious trees generated with a branch and bound algorithm in PAUP 3.1.1. Trees were obtained from aligned sequences of the 5.8S gene and flanking ITS1 and ITS2 regions (15 steps, CI = 0.8, RI = 0.786). Bootstrap values above 50 % are shown. A *Fusarium subglutinans* sequence (EMBL accession number X94167) was used as outgroup.

Additional differences were observed in the ITS1 region of the four species in the *Cy. candelabrum* complex. Single base pair substitutions in the ITS2 region at base pairs could distinguish *Cy. floridanum* from the other species' sequences, while a similar single base difference could differentiate the four species in the *Cy. candelabrum* complex from *Cy. scoparium* and *Cy. floridanum*. Further single base deletions and substitutions distinguished all species on the basis of sequence dissimilarity. Accordingly, a phylogenetic tree was produced using PAUP analysis (Swofford 1993). Figure 3 shows one of the four most parsimonious trees obtained by branch and bound analysis of the informative sites of the 5.8S and flanking ITS1 and ITS2 DNA regions for the six species mentioned above. All four most parsimonious trees indicated a closer relationship between the sequences of *Cy. insulare* and those of *Cy. scoparium* and *Cy. floridanum*. The exact relationships between the other species were ambiguous.

Morphological comparisons

Several morphological characters were studied. This included the shape and diameter of the terminal vesicles extending from the conidiophore stipes, conidial size, conidiophore branching pattern, ascospore shape, size, perithecial colour, anatomy, morphology, and cultural characteristics.

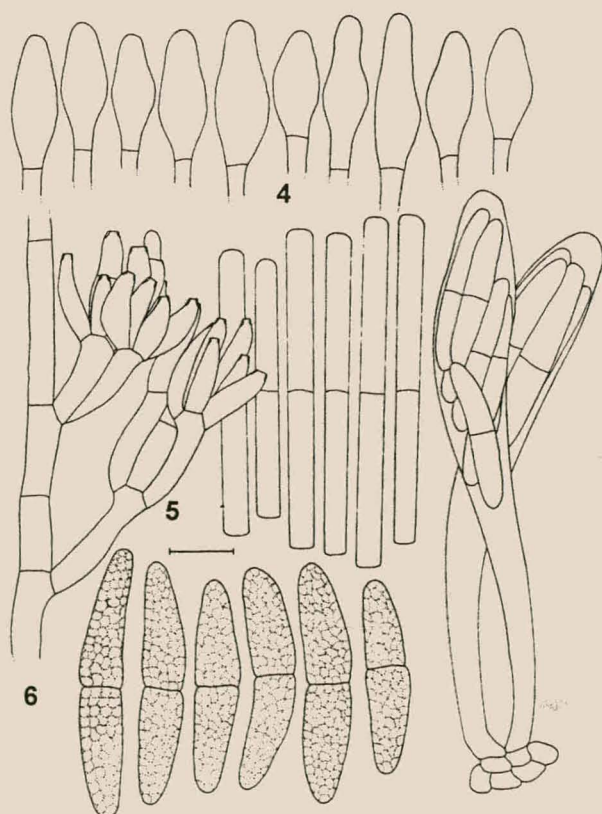
The morphological similarities of the anamorph and teleomorph states corresponded well with the results obtained in the mating studies, and grouped isolates into four distinct groups. The four groups identified based on these features were further supported by their distinct DNA sequences, which led us to conclude that they represent four biological species, which are subsequently described below.

Species descriptions

Calonectria pauciramosa, C.L. Schoch & Crous sp. nov.

Figs. 4-11

Anamorph. *Cylindrocladium pauciramosum* C.L. Schoch & Crous sp. nov.

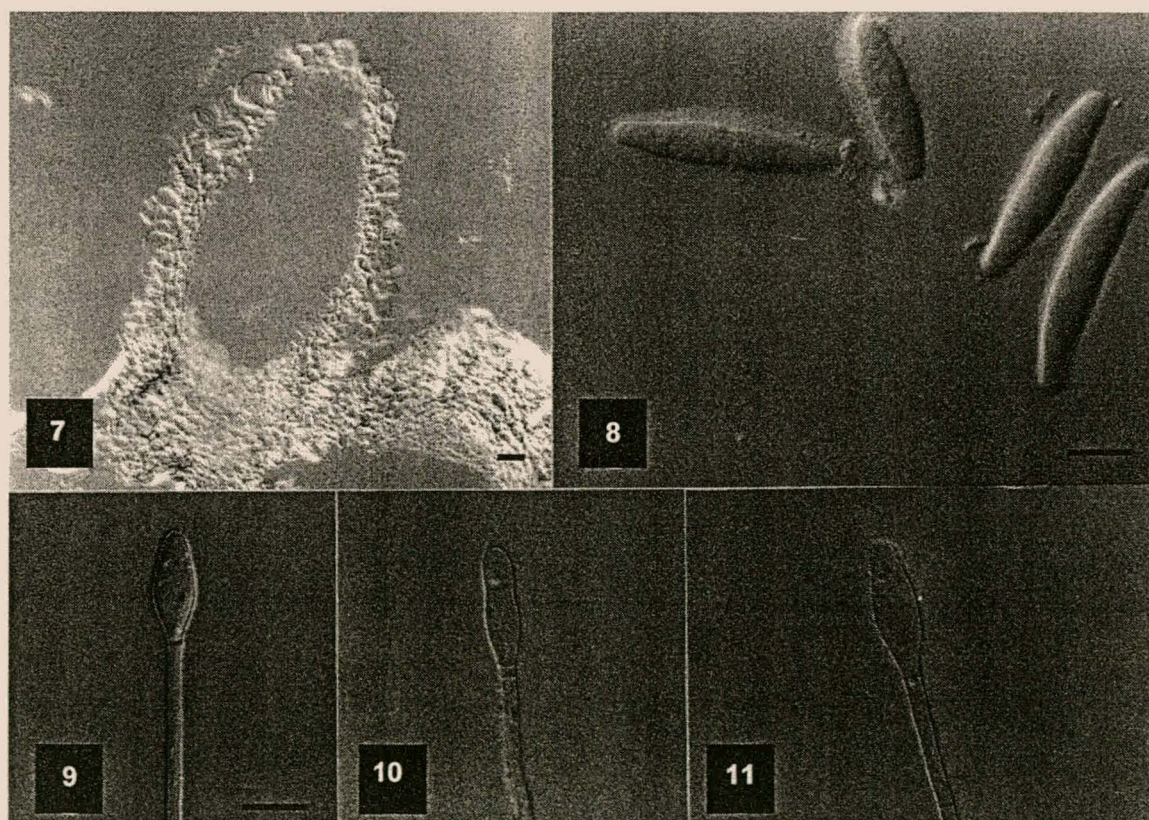


Figs. 4-6. *Calonectria pauciramosa* and its anamorph *Cylindrocladium pauciramosum*. 4. Terminal vesicles on stipe extensions. 5. Conidiophore and conidia. 6. Asci and ascospores. Bar = 10 μ m.

Etymology. Refers to the relatively low number of conidiophore branches in the species.

Holotypes. BRAZIL × SOUTH AFRICA. BRAZIL. BAHIA: Nursery, *Eucalyptus* sp., Jul. 1990, A. C. Alfenas; Knysna, soil, Nov. 1994, P. W. Crous, heterothallic mating of STE-U 1670 (PREM 55753 anamorph) × STE-U 971 (PREM 55752 anamorph holotype), Apr. 1997 C. L. Schoch (PREM 55754 teleomorph holotype).

Description. Perithecia subglobosa ad ovoidea, 250-400 µm alta, 170-300 µm lata, crocea ad rubro-brunnea, pariete exteriori verrucosa, ostiolo papillato. Asci clavati, in stipitem longum tenuem gradatim angustatae, 70-140 × 8-25 µm, 8-spori. Ascospores hyalinae, fusiformes, 1-septatae, nihil vel leviter ad septum constrictae, (30-)33-38(-40) × 6-7(-8) µm. Filum septatum, hyalinum (120-)180(-230) µm, in vesiculam obpyriformam ad late ellipsoidam (5-)7-9(-11) µm diam terminans. Conidia cylindrica, hyalina, 1-septata, apicibus obtusis, (30-)45-55(-60) × (3.5-)4-5 µm. Microconidiophora ignota.



Figs. 7-11. *Calonectria pauciramosa* and its anamorph *Cyliandrocladium pauciramosum*. 7. Vertical section through a perithecium. 8. Ascospores. 9-11. Terminal vesicles. Bars = 10 µm.

Perithecia orange to red-brown, subglobose to ovoid, 250-400 µm high, 170-300 µm wide, turning dark red in 3% KOH; ostiole papillate. Perithecia rough-walled, wall consisting of two layers: outside layer of *textura globulosa*, 20-50 µm wide; inner

layer of *textura angularis*, 5-10 μm wide, outer cells 40-55 x 15-35 μm ; hymenial layer of *textura prismatica*, hyaline, 5-10 μm wide; perithecial base up to 100 μm wide, consisting of dark red, angular cells. *Asci* 8-spored, clavate, 70-140 x 8-25 μm , tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, fusoid with rounded ends, straight to slightly curved, 1-septate, not or slightly constricted at the septum, (30-)33-38(-40) x 6-7(-8) μm . *Macroconidiophores* comprised of a stipe, a sterile elongation and a penicillate arrangement of fertile branches. Stipe septate, (120-)180(-230) μm long, terminating in an obpyriform to broadly ellipsoidal vesicle, (5-)7-9(-11) μm diam; primary branches aseptate or 1-septate, 12-45 x 5-6 μm ; secondary branches aseptate, 15-20 x 5 μm , and tertiary branches aseptate, 12-15 x 5 μm , each terminal branch producing 2-6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10-13 x 2.5-4 μm , apex with minute periclinal thickening and inconspicuous collarete. *Conidia* cylindrical, rounded at both ends, straight, (30-)45-55(-60) x (3.5-)4-5 μm , 1-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime. *Microconidiophores* not observed. *Chlamydospores* dark brown, thickened, formed in extensive numbers throughout the medium, and aggregated to form microsclerotia.

Cultures. Colony colour (underneath) 13i fulvous, (surface) 13i sienna with abundant white aerial mycelia. Colony margin irregular, with extensive chlamydospores and sparse sporulation on aerial mycelia. Colonies obtaining a radius of 17-20 mm diam on MEA after 6 d in dark at 25°C.

Cardinal temperatures for growth. Minimum above 5°C, maximum below 35°C, optimum 25°C. This is both a high and low temperature species, growing below 5°C, and above 30°C.

Substrate. *Acacia cyclops*, *Azalea* sp., *Eucalyptus* spp., *Fragaria* sp., *Protea* sp., *Rhododendron* sp., soil.

Distribution. Australia, Brazil, Colombia, Mexico, South Africa.

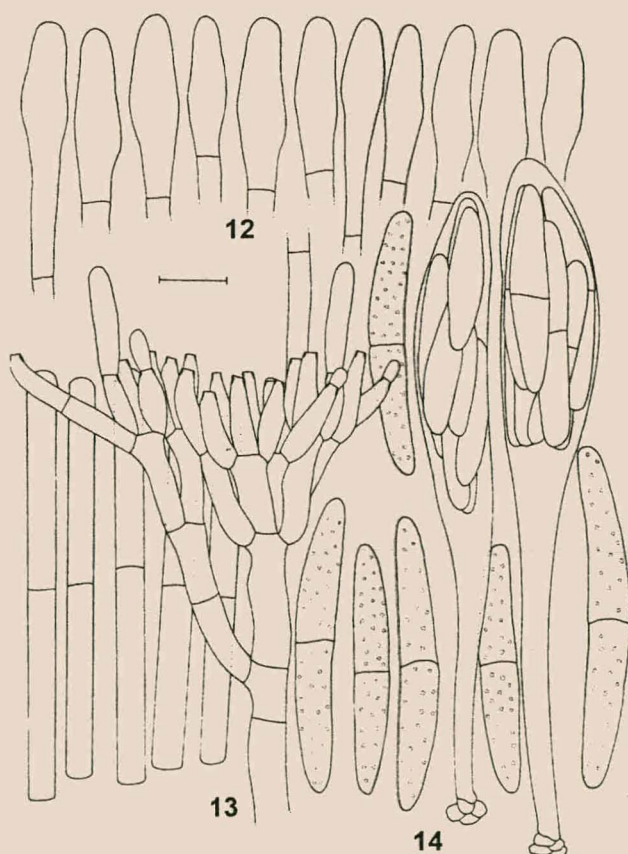
Additional cultures examined. AUSTRALIA. QUEENSLAND: Locality unknown, strawberry, 1991, *D. Hutton* (N167/91 = STE-U 1691; N335/91 = STE-U 1692). BRAZIL. BAHIA: Vivieros, *Eucalyptus* sp., Jul. 1990, *A. C. Alfenas* (UFV 25 = STE-U 1670; UFV 27 = STE-U 1671). SANTA CATARINA: Florianópolis, soil, Apr. 1994, *M. J. Wingfield* (STE-U 911-913, 923-925). COLOMBIA. CÓRDOBA: La Selva, Jun. 1995, *M. J. Wingfield* (STE-U 1160-1163). MEXICO. VERACRUZ: Catemaco,

Laguna Encantada, soil, Apr. 1994 *M. J. Wingfield* (STE-U 951). SOUTH AFRICA. KWAZULU-NATAL: Kwambonambi, *Eucalyptus grandis* seedlings, Feb. 1990, *P. W. Crous* (STE-U 247, 249, 256, 257, 271, 274, 344, 346); *Eucalyptus grandis*, Oct. 1995, *P. W. Crous* (STE-U 1239); Pietermaritzburg, *Eucalyptus nitens*, Mar. 1990, *P. W. Crous* (STE-U 391); WESTERN CAPE: *Acacia cyclops*, Jul. 1990, *M. Morris* (CMM 953 = STE-U 1693); George, *Azalea* bushes, Feb. 1993, *S. Lamprecht* (STE-U 575); Knysna, soil, Nov. 1994, *P. W. Crous* (STE-U 971, 972); MPUMALANGA: Kruisfontein, *Eucalyptus grandis* trunk, Sept. 1989, *P. W. Crous* (STE-U 138, 143); Sabie, soil, Feb. 1990, *P. W. Crous* (STE-U 356, 358); Klipkraal, *Eucalyptus grandis* seedlings, Feb. 1990, *P. W. Crous* (STE-U 286-288); Witrivier, *Azalea* sp., May 1990, *S. Lamprecht* (STE-U 379, 380); NORTHERN PROVINCE: Piet Retief, pine cuttings, Nov. 1994, *P. W. Crous* (STE-U 958, 959); Tzaneen, *Eucalyptus grandis* seedlings, Feb. 1990, *P. W. Crous* (STE-U 282-284), *Eucalyptus grandis* cuttings, Jun. 1990, *S. de Buisson* (STE-U 416, 417).

Calonectria scoparia Peerally, Mycotaxon 40: 341 (1991).

Figs. 12-18

Anamorph. *Cylindrocladium candelabrum* Viégas, Bragantia 6: 370 (1946).



Figs. 12-14. *Calonectria scoparia* and its anamorph *Cylindrocladium candelabrum*. 12. Terminal vesicles on stipe extensions. 13. Conidiophore and conidia. 14. Asci and ascospores. Bar = 10 μ m.

Holotypes. BRAZIL. BAHIA. Picadao, Conceicao de Barra, *Eucalyptus grandis*, Apr. 1992, A. C. Alfenas & F. A. Ferreira (PREM 51045 neotype of teleomorph; Crous et al 1993a); Copener, *Eucalyptus* sp., A. C. Alfenas, PREM 51044 (neotype of anamorph; Crous et al 1993a), culture ex-type PPRI 4135.

Description. *Perithecia* red-brown, subglobose to ovoid, 350-450 μm high, 300-350 μm wide, turning dark red in 3% KOH, frequently in clusters of 3-4; ostiole papillate. *Perithecia* rough-walled, wall consisting of two layers: outside layer of *textura globulosa*, 50-100 μm wide; inner layer of *textura angularis*, 5-10 μm wide, outer cells 35-45 x 18-30 μm ; hymenial layer of *textura prismatica*, hyaline, 5-10 μm wide; perithecial base up to 150 μm wide, consisting of dark red, angular cells. *Asci* 8-spored, clavate, 70-130 x 7-15 μm , tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, fusoid with rounded ends, straight to slightly curved, 1-septate, not to slightly constricted at the septum, (40-)45-50(-60) x 5-6 μm ; becoming 3-septate once discharged. *Macroconidiophores* comprised of a stipe, a sterile elongation and a penicillate arrangement of fertile branches. Stipe septate, (100-)170(-220) μm long, terminating in an ellipsoidal to narrowly obpyriform vesicle, (5-)6-7(-8) μm diam; primary branches aseptate or 1-septate, 20-45 x 4-5 μm ; secondary branches aseptate, 15-25 x 4-5 μm , tertiary branches aseptate, 15-20 x 4-5 μm , and quaternary branches aseptate, 10-15 x 4-5 μm , each terminal branch producing 2-6 phialides; phialides dolliiform to reniform, hyaline, aseptate, 10-20 x 3-4 μm , apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (45-)58-68(-80) x 4-5(-6) μm , 1-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime. *Microconidiophores* not observed. *Chlamydospores* dark brown, thickened, formed in extensive numbers throughout the medium, and aggregated to form microsclerotia.

Cultures. Colony colour (underneath) 13i fulvous, (surface) 13i sienna. Colony margin irregular with sparse to moderate aerial mycelia, and extensive chlamydospores. Colonies obtaining a radius of 12-17 mm diam on MEA after 6 d in the dark at 25°C.

Cardinal temperatures for growth. Minimum above 5°C, maximum below 35°C, optimum 25°C. This is both a high and low temperature species, with medium sporulation on aerial mycelium.

Substrate. *Eucalyptus* spp., *Luma* sp., soil.

Distribution. Brazil, Venezuela.

Additional specimens deposited. BRAZIL. BAHIA: Vivieros, soil, heterotallic mating of STE-U 1675 (PREM 55755 anamorph) × STE-U 1677 (PREM 55756 anamorph), Apr. 1997, C. L. Schoch, (PREM 55757 teleomorph).



Figs. 15-18. *Calonectria scoparia* and its anamorph *Cyindrocladium candelabrum*. 15. Vertical section through a perithecium. 16. Ascospores. 17, 18. Terminal vesicles. Bars = 10 μm.

Additional cultures examined. BRAZIL. AMAZONAS: Locality unknown, *Eucalyptus* sp., 1991, A. C. Alfenas (UFV 117 = STE-U 1675; UFV 118 = STE-U 1676; UFV 121 = STE-U 1677; UFV 122 = STE-U 1678; UFV 126 = STE-U 1679; UFV 128 = STE-U 1680; UFV 129 = STE-U 1681; UFV 130 = STE-U 1682; UFV 132 = STE-U 1683); *Eucalyptus* sp., 1991, J. C. Dianese (D1038 = STE-U 1684); Belém, *Eucalyptus* sp., Feb. 1990, M. J. Wingfield (STE-U 313); BAHIA: Copener, *Eucalyptus* sp., Jul. 1990, A. C. Alfenas (UFV 63 = STE-U 1674); Vivieros, *Eucalyptus* sp., Jul. 1990, ACA: (UFV 29 = STE-U 1672); MINAS GERAIS: Ipatinga, *Eucalyptus* sp., Jul. 1990, A. C. Alfenas (UFV 45 = STE-U 1673); Bocaiúva, *Eucalyptus* sp., Jul. 1990, A. C. Alfenas (UFV 170 = STE-U 1685); Bom Despacho, *Eucalyptus* sp., Jul. 1990, A. C. Alfenas (UFV 172 = STE-U 1686); SÃO PAULO: São Paulo, *Eucalyptus* cuttings, Mar. 1993, P. W. Crous (STE-U 586, 594, 597, 600-602, 604, 605). VENEZUELA. Locality unknown, soil, Jun. 1995, M. J. Wingfield, (STE-U 1183).

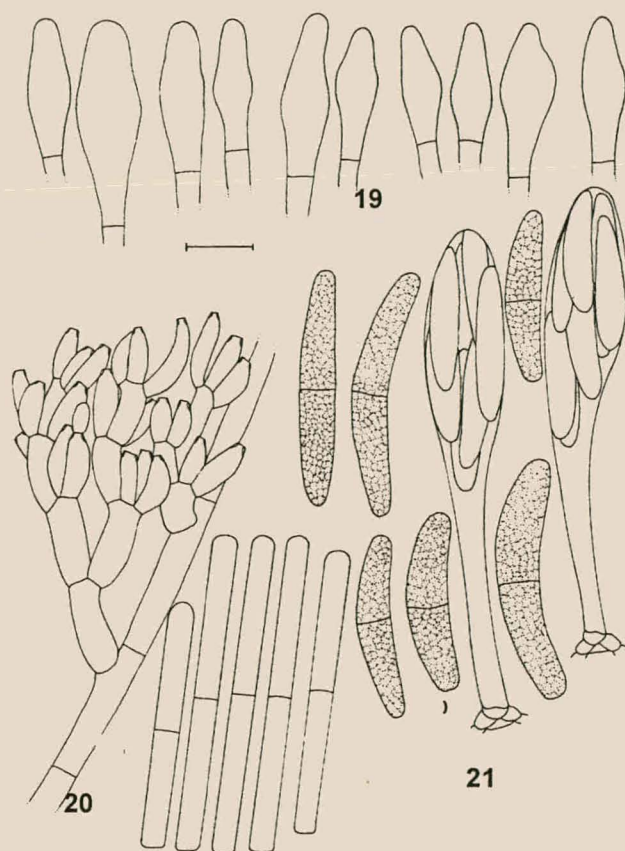
Calonectria insularis, C.L. Schoch & Crous sp. nov.

Figs. 19- 25

Anamorph. *Cylindrocladium insulare*, sp. nov.

Etymology. In reference to its geographic distribution.

Holotypes. MADAGASCAR: Tamatave, soil, Apr. 1997, *P. W. Crous*, heterothallic mating of STE-U 766 (PREM 55758 anamorph holotype) × STE-U 768 (PREM 55759 anamorph), Apr. 1997, *C. L. Schoch*, (PREM 55760 teleomorph holotype).



Figs. 19-21. *Calonectria insularis* and its anamorph *Cylindrocladium insulare*. 19. Terminal vesicles on stipe extensions. 20. Conidiophore and conidia. 21. Asci and ascospores. Bar = 10 μ m.

Discriptions. Perithecia subglobosa ad ovoidea, 350-450 μ m alta, 300-350 μ m lata, crocea ad rubra, pariete exteriore verrucosa, ostiolo papillato. Asci clavati, in stipitem longum tenuem gradatim angustatae, 70-120 x 7-18 μ m, 8-spori. Ascosporae hyalinae, fusiformes, 1-septatae, ad septum nihil constrictae, (27-)30-36(-42) x 5-6(-7) μ m. Ascosporae evolentes usque ad constrictae dismissae ab asco. Filum septatum, hyalinum (110-)160(-250) μ m, in vesiculam obpyriformam ad late ellipsoidam (4-)7-10(-13) μ m diam terminans. Conidia cylindrica, hyalina, 1-septata, apicibus obtusis, (33-)40-50(-60) x 3.5-4 μ m. Microconidiophora ignota.

Perithecia orange to red, subglobose to ovoid, 350-450 µm high, 300-350 µm wide, turning dark red in 3% KOH; ostiole papillate. *Perithecia* rough-walled, wall consisting of two layers: outside layer of *textura globulosa*, 40-80 µm wide; inner layer of *textura angularis*, 5-10 µm wide, outer cells 25-45 x 20-35 µm; hymenial layer of *textura prismatica*, hyaline, 5-10 µm wide; perithecial base up to 100 µm wide, consisting of dark red, angular cells. *Asci* 8-spored, clavate, 70-120 x 7-18 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, fusoid with rounded ends, straight to slightly curved, 1-septate, not constricted at the septum, becoming constricted once discharged, (27-)30-36(-42) x 5-6(-7) µm. *Macroconidiophores* comprised of a stipe, a sterile elongation and a penicillate arrangement of fertile branches. Stipe septate, (110-)160(-250) µm long, terminating in an obpyriform to broadly ellipsoidal vesicle, (4-)7-10(-13) µm diam; primary branches aseptate or 1-septate, 10-45 x 4-5 µm; secondary branches aseptate, 10-25 x 4-5 µm, tertiary branches aseptate, 10-17 x 4-5 µm, and quaternary branches aseptate, 10-12 x 4-5 µm, each terminal branch producing 2-6 phialides; phialides doliform to reniform, hyaline, aseptate, 9-14 x 3-5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (33-)40-50(-60) x 3.5-4 µm, 1-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime. *Microconidiophores* not observed. Dark brown, thickened *chlamydospores* formed in extensive numbers throughout the medium, and aggregated to form microsclerotia.

Cultures. Same characteristics as *Cy. pauciramosum* with colonies obtaining a radius of 18-23 mm diam on MEA after 6 d in the dark at 25°C.

Cardinal temperatures for growth. Minimum above 15°C, maximum above 35°C, optimum 25-30°C. This is a high temperature species.

Substrate. *Acacia* sp., *Auracaria heterophylla*, *Medicago sativa*, *Persea americana*, *Pisum sativum*, *Eucalyptus* sp., soil.

Distribution. Brazil, Hawaii, Indonesia, Madagascar, Malaysia, Mauritius, Mexico.

Additional cultures examined. BRAZIL. AMAZONAS: Belém, soil, Apr. 1993, *M. J. Wingfield* (STE-U 616, 620, 625, 626). INDONESIA. SUMATRA: Sei Kobaro, *Acacia mangium* rhizosphere, Jan. 1994, *A. C. Alfenas* (STE-U 722). MADAGASCAR. Tamatave, soil, Apr. 1994, *P. W. Crous* (STE-U 766, 768). MALAYSIA. MALAY

PENINSULA: Kemasik, *Acacia* sp., Dec. 1995, *M. J. Wingfield* (STE-U 1281, 1282). MAURITIUS. Rivière Noire, soil, Apr. 1996, *H. Smith* (STE-U 1473, 1474). Pampalmousses, soil, Apr. 1996, *H. Smith* (STE-U 1475). MEXICO. VERACRUZ: Conejos, Puente Nacional, soil, Apr. 1994, *M. J. Wingfield* (STE-U 952, 954). U.S.A. HAWAII: Locality unknown, *Medicago sativa*, 1981, *M. Aragaki* (A 890 = STE-U 1687); *Auracaria heterophylla*, 1987, *M. Aragaki* (A 1570 = STE-U 1688); *Pisum sativum*, 1988, *M. Aragaki* (A 1823 = STE-U 1689); *Persea americana*, 1988, *M. Aragaki* (A 1853 = STE-U 1690).



Figs. 22-25. *Calonectria insularis* and its anamorph *Cylindrocladium insulare*. 22. Vertical section through a perithecium. 23. Ascospores. 24, 25

Calonectria mexicana, C.L. Schoch & Crous sp. nov.

Figs. 26-35

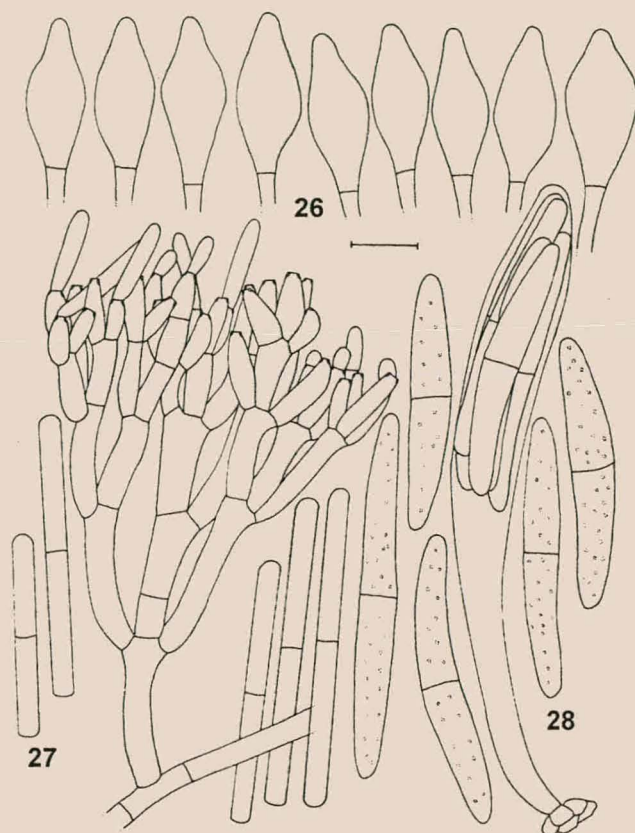
Anamorph. *Cylindrocladium mexicanum*, sp. nov.

Etymology. In reference to its country of origin.

Holotypes. MEXICO. YUCATAN: Uxmal, soil., Apr. 1994 *M. J. Wingfield*; HOLPECHÉN: Campeche, soil., Apr. 1994, *M. J. Wingfield*, heterothallic mating of STE-U 927 (PREM 55761 anamorph holotype) × STE-U 941 (PREM 55762 anamorph), Apr. 1997, *C. L. Schoch* (PREM 55763 teleomorph holotype).

Descriptions. Perithecia subglobosa ad ovoidea, 400-450 µm alta, 350-450 µm lata, crocea ad rubra, pariete exteriori verrucosa, ostiolo papillato. Asci clavati, in stipitem longum tenuem gradatim angustatae, 70-120 x 10-20 µm, 8-spori. Ascosporae hyalinae, fusiformes, 1-septatae, nihil vel leviter constrictae ad septum,

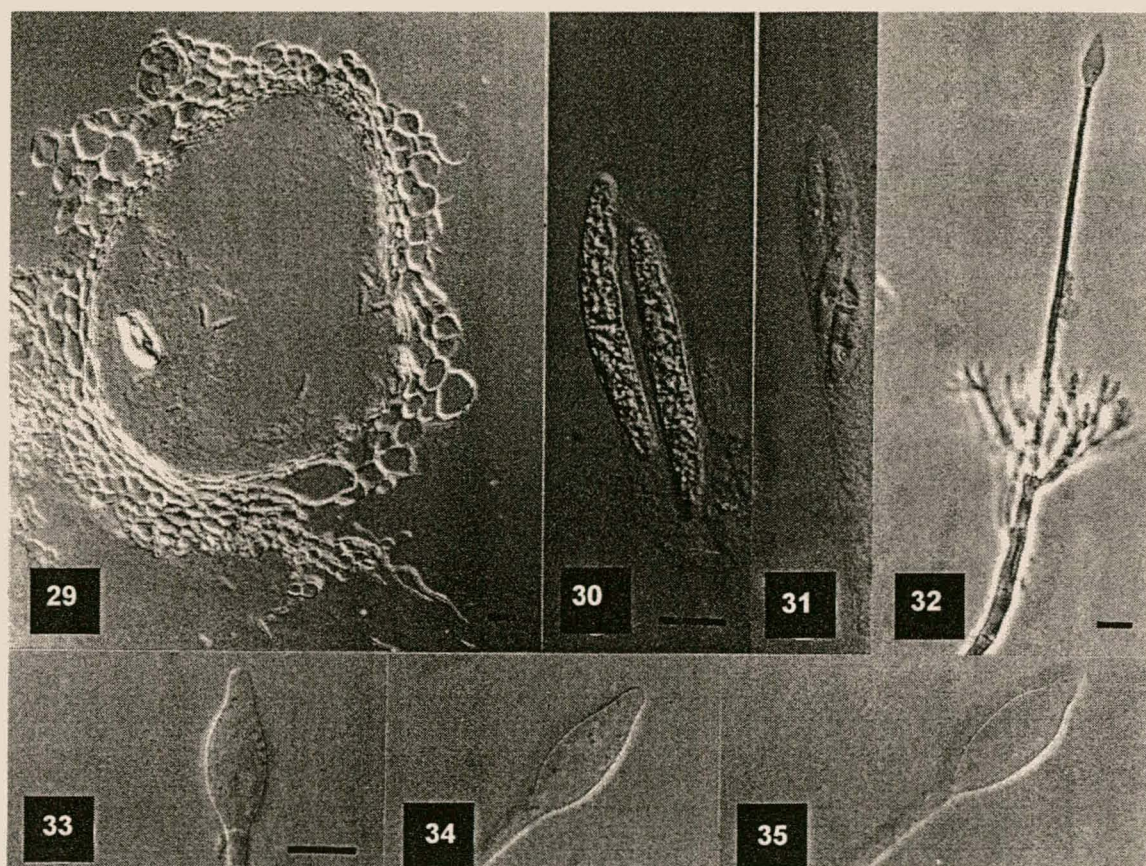
(35-)40-55(-65) x 5-6(-7) μm . Ascoporaevolentes usque ad tres septa dismissae ab asco. Filum septatum, hyalinum (160-)180(-250) μm , in vesiculam late ellipsoidam apicibus papillatis (7-)8-10(-12) μm diam terminans. *Conidia* cylindrica, hyalina, 1-septata, apicibus obtusis, (35-)40-48(-52) x 3-4(-4.5) μm . *Microconidiophora* ignota.



Figs. 26-28. *Calonectria mexicana* and its anamorph *Cylindrocladium mexicanum*. 26. Terminal vesicles on stipe extensions. 27. Conidiophore and conidia. 28. Asci and ascospores. Bar = 10 μm .

Perithecia orange to red, subglobose to ovoid, 400-450 μm high, 350-450 μm wide, turning dark red in 3% KOH; ostiole papillate. *Perithecia* rough-walled, wall consisting of two layers: outside layer of *textura globulosa*, 35-90 μm wide; inner layer of *textura angularis*, 5-15 μm wide, outer cells 20-35 x 20-30 μm ; hymenial layer of *textura prismatica*, hyaline, 5-10 μm wide; perithecial base up to 100 μm wide, consisting of dark red, angular cells. *Asci* 8-spored, clavate, 70-120 x 10-20 μm , tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, fusoid with rounded ends, straight to slightly curved, 1-septate, not or slightly constricted at the septum, (35-)40-55(-65) x 5-6(-7) μm ; becoming 3-septate once discharged. *Macroconidiophores* comprised of a stipe, a sterile elongation and a penicillate arrangement of fertile branches. Stipe septate, (160-)180(-250) μm long, terminating in a broadly ellipsoidal vesicle with a papillate apex, (7-)8-10(-12) μm diam; primary branches aseptate or 1-septate, 17-45 x 4-6 μm ; secondary

branches aseptate, 15-25 x 4-5 μm , tertiary branches aseptate, 11-17 x 3-5 μm , and quaternary branches aseptate, 10-15 x 2.5-4 μm , each terminal branch producing 2-6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7-16 x 3-4 μm , apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (35-)40-48(-52) x 3-4(-4.5) μm , 1-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime. *Microconidiophores* not observed. *Chlamydospores* dark brown, thickened, formed in extensive numbers throughout the medium, and aggregated to form microsclerotia.



Figs. 29-35. *Calonectria mexicana* and its anamorph *Cyindrocladium mexicanum*. 29. Vertical section through a perithecium. 30. Ascospores. 31. Asci. 32. Conidiophore with extending stipe and terminal vesicle. 33-35. Terminal vesicles. Bars = 10 μm .

Cultures. Colony colour (underneath) 13b – 13i (orange to sienna), (surface) similar as underneath with moderate white aerial mycelia. Colony margin irregular with extensive chlamydospores and sparse sporulation on aerial mycelium. Colonies obtaining a radius of 17-20 mm diam on MEA after 6 d in the dark at 25°C.

Cardinal temperatures for growth. Minimum above 10°C, maximum above 35°C, optimum 25-30°C. This is both a high and low temperature species.

Substrate. soil.

Distribution. Mexico.

Additional cultures examined. MEXICO. CAMPECHE: Holpechén, soil, Apr. 1994, *M. J. Wingfield* (STE-U 941-943, 966, 967); YUCATAN: Uxmal, soil, Apr. 1994, *M. J. Wingfield* (STE-U 926-928, 944-946).

Discussion

This study was initiated in order to investigate the morphological variability observed within the *Cy. candelabrum* species complex. Mating studies revealed the existence of four distinct mating populations in this complex. These findings were further supported by differences in morphology, and sequence data. In accordance with the biological species concept, different species were therefore proposed for each mating population.

Previous mating studies between isolates of *Cy. scoparium* and *Cy. candelabrum* showed these species to be genetically isolated (Crous et al 1993a). Within the *Cy. candelabrum*-complex, however, prominent differences were observed when perithecia of South African x South African, or South African x Brazilian matings were compared with some Brazilian x Brazilian matings. In light of the distribution data of some of these species (*Cy. pauciramosum* and *Cy. candelabrum*) as circumscribed in the present study, it is obvious that the variation observed by Crous et al (1993a) can now be ascribed to different biological species. In light of the results presented here, previous mating groups observed in *Cy. candelabrum* (as *Cy. scoparium*; Ribeiro 1978), suggest that yet other, undescribed biological species could exist in this complex. Recent molecular work done in another homothallic species complex, *Cy. florianum* (Victor et al 1997), suggests that this aggregation of distinct biological taxa in species complexes is much more common in *Cylindrocladium* than expected earlier.

The high proportion of successful matings obtained in the present study, and recently by Crous et al (1998) in *Cy. ovatum*, can possibly be ascribed to the fact that these matings were conducted at 22°C, compared to previous studies that used 15 and 25°C as optimum temperature. Within each species, however, isolates showed

varying degrees of success in mating with opposing mating types. For example, in *Cy. pauciramosum* STE-U 138 mated only with two other opposing mating type strains, while in *Cy. candelabrum* STE-U 1678 mated successfully in all instances. Age of isolates as well as differences in their female fertility could account for this variation. It appears that *Cy. pauciramosum* and *Cy. insulare* are largely allopatric in character, with isolates available from various localities.

Sequence data can quantify relatedness among taxa, and is commonly used to clarify different taxonomic questions (Viljoen et al 1993, Rehner & Samuels 1995). The sequences of the ITS1 and ITS2 flanking regions of the 5.8S ribosomal gene indicated small, but consistent differences between the species proposed in this study. Although a high degree of sequence variation in this region has been reported before (Chambers et al 1986), a low amount of variation was observed between the *Cylindrocladium* species examined in the present study. Within a biological species no variation could be observed at all. Even in the case of *Cy. insulare*, identical sequences were observed for isolates from disparate geographic areas like Madagascar, Mexico and Brazil. When compared to a similar situation in *Gibberella fujikuroi*, where several mating populations exist between isolates with similar morphological features (Leslie 1995), the high relatedness in the *Cy. candelabrum* complex becomes more evident. However, sequences of the 5.8S gene and ITS1 and ITS2 flanking regions proved problematic in differentiating the different mating populations in the *Gibberella fujikuroi* complex (Waalwijk et al 1996). Although the species in this study could be differentiated using sequence results, further consideration will have to be given to other, more variable DNA regions. Studies conducted in the hypocrealean genus *Fusarium* (O'Donnell 1996), could prove useful in this regard.

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3. Recombination in *Cylindrocladium scoparium* and phylogeny with other heterothallic small-spored *Cylindrocladium* species*

Abstract

The *Cylindrocladium scoparium* cultures studied were isolated from several hosts in the U.S.A. Isolates were mated in all combinations, and one successful mating was selected to establish whether recombination occurred. RAPD and mating type data of parental isolates and progeny confirmed *Cy. scoparium* to have a biallelic heterothallic mating system. Furthermore, to determine the phylogeny of *Cy. scoparium* with several morphologically similar *Cylindrocladium* spp., DNA sequences of the ribosomal 5.8S gene and the flanking internal transcribed spacers (ITS), as well as part of the high mobility group (HMG) box (forming part of the *MAT-2* mating type gene) and the β -tubulin gene, were analysed. Maximum parsimony yielded concordant trees for all three data sets. These data supported the morphological and biological species concepts proposed for *Cy. scoparium* and other, similar, small-spored *Cylindrocladium* spp.

Introduction

Cylindrocladium scoparium Morgan is the type species of the anamorph genus *Cylindrocladium* Morgan (Morgan 1892). Members of this genus have *Calonectria* De Not. teleomorphs, are ubiquitous plant pathogens and have been isolated mainly in tropical and subtropical regions of the world. *Cylindrocladium scoparium* (teleomorph *Calonectria morganii* Crous et al) has reportedly been associated with a wide range of disease symptoms, including damping off, root rot, cutting rot, stem cankers, leaf-spot and seedling blight (Cordell & Rowan 1975). Although this species has been reported from over 30 plant families (Booth & Gibson 1973, French & Menge 1978, Peerally 1991, Waipara et al 1996), recent data (Part 2) suggest that many of these records were incorrectly ascribed to *Cy. scoparium*.

* Submitted: Schoch CL, Crous PW, Cronwright G, Witthuhn RC, El-Gholl NE, Wingfield B. 1999. Recombination in *Cylindrocladium scoparium* and phylogeny with other heterothallic small-spored *Cylindrocladium* species. Mycologia.

The main taxonomic criteria used for the identification of *Cylindrocladium* species are conidial and ascospore size and septation, vesicle shape and diameter, and perithecial morphology. Although the reliability of the terminal vesicle as criterion for species identification has been questioned by some workers (Hunter & Barnett 1978, Rossman 1983), Crous et al (1992) showed that this is useful when studied under controlled conditions on carnation-leaf agar (CLA) (Fisher et al 1982).

However, uncertainty still exists regarding the identification of *Cy. scoparium*, and it has frequently been confused with other species with 1-septate, small conidia. These include *Cy. ovatum* El-Gholl et al (ovoid vesicles), *Cy. floridanum* Sobers & C.P. Seym. (sphaeropedunculate vesicles) and *Cy. candelabrum* Viégas (obpyriform vesicles). Victor et al (1997) compared isolates of these taxa and showed that they represent different species. The latter study also confirmed the existence of genetically distinct groups among isolates of *Cy. floridanum*, which was initially reported by Jeng et al (1997). A similar situation has also been found to exist in other species complexes such as *Cy. gracile* (Crous et al 1995, 1997a, b) and *Cy. candelabrum* (Part 2).

Cylindrocladium scoparium has been reported from various areas worldwide, including Africa (Doidge 1950, Darvas et al 1978, Botha & Crous 1992), South America (Palmucci et al 1996, Tozetto & Ribeiro 1996), Europe (Overmeyer et al 1996, Polizzi & Azzaro 1996), Asia (Mohanan & Sharma 1985, Srinivasan & Gunasekaran 1995) and New Zealand (Waipara et al 1996). However, the presence of *Cy. scoparium* has only been confirmed from North America and Brazil (Crous et al 1993a), and many of the isolates discussed in the previously mentioned reports have proven to be the newly described *Cy. pauciramosum* C.L. Schoch & Crous, which forms part of the *C. candelabrum* species complex (Part 2).

The low mating frequency reported in previous studies of *Cy. scoparium* (Crous et al 1993a) and related species (Victor et al 1997) have complicated studies in these fungi by limiting the use of mating testers for species identification. Overmeyer et al (1996) reported only a single mating between mating type tester strains obtained from the American Type Culture Collection (ATCC). Furthermore, no successful matings were obtained with any of the additional thirty-two strains isolated from various hosts in Germany. High success rates were, however, recently obtained for matings done with *Cy. ovatum* (Crous et al 1998) and species in the *Cy.*

candelabrum species complex (Part 2). These results confirmed that these species have biallelic, heterothallic mating systems.

A similar mating system was originally described for *Cy. scoparium* (Crous et al 1993a). Results obtained by Overmeyer et al (1996) indicated a different scenario, because only one parent was reported to contribute to the genetic makeup of progeny. However, as so few matings with *Cy. scoparium* have proven successful in the past (Crous et al 1993a, Overmeyer et al 1996, Victor et al 1997), it was decided to also employ molecular techniques to provide more information on whether recombination occurred or not.

Random amplified polymorphic DNA (RAPD) is a technique that has been applied to answer various genetically oriented questions. Previous studies have applied RAPD data in order to show recombination among agricultural crops (Echt et al 1992) and fungal pathogens (Nicholson et al 1995, Campbell et al 1999). This technique was therefore chosen to verify whether recombination occurred during matings of *Cy. scoparium*.

The phylogenetic relatedness of various *Cylindrocladium* species as suggested by morphological features is still largely uncertain. Several molecular characters have previously been used to analyse relationships among *Cylindrocladium* spp. These include protein profiles (Crous et al 1993b), RAPDs (Victor et al 1997) and Restriction fragment length polymorphisms (RFLP) (Crous et al 1997b). Previous results by Jeng et al (1997) showed that isolates of *Cy. scoparium* and *Cy. floridanum* could be distinguished by DNA sequence analysis of the 5.8S ribosomal RNA gene and flanking internally transcribed spacers (ITS). More recently data obtained from mating studies were combined with the analysis of ITS sequences in the *Cy. candelabrum* species complex (Part 2), emphasising the low number of informative characters available in the DNA sequence data of the ITS region.

In a study aimed at differentiating species in the *Gibberella fujikuroi* species complex, O'Donnell et al (1998) employed sequence data of the nuclear 28S rDNA, mitochondrial small subunit (SSU) and β -tubulin gene. From these data it was shown that the β -tubulin gene yielded the most variation of all areas sequenced, making it useful for determining phylogeny in newly diverged groups. Degenerate primers based on conserved regions in the HMG (high mobility group) box in the *mt a-1*

mating type gene of *Neurospora crassa* Shear & B.O. Dodge have successfully been employed to amplify partial *MAT-2* (*mt a-1*) sequences from other species in the pyrenomycetes (Arie et al 1997, Turgeon 1998, Witthuhn et al 1999).

Based on the clear advantages of these techniques to separate closely related species, the aim of the present study was to use these sequences to infer the phylogeny of *Cy. scoparium* and other small-spored, heterothallic *Cylindrocladium* species.

Materials and Methods

Isolates

Cylindrocladium scoparium isolates studied were either isolated from symptomatic material, or obtained from the American Type Culture Collection (ATCC 46300 and ATCC 38227) (Table I). All isolates were identified using the methods reported by Crous et al (1997b) and those in Part 2.

Table I. Isolates used in this study.

Species	Culture no.	Collector	Host	Origin
<i>Cy. scoparium</i>	STE-U 496	A.C. Alfenas	Unknown	U.S.A.
	STE-U 497	A.C. Alfenas	Unknown	U.S.A.
	STE-U 654	A.C. Alfenas	Unknown	U.S.A.
	STE-U 655	A.C. Alfenas	Unknown	U.S.A.
	STE-U 1720	N.E. El-Gholl	<i>Rosa</i> sp.	Florida, U.S.A.
	STE-U 1721	N.E. El-Gholl	<i>Conocarpus erectus</i>	Florida, U.S.A.
	STE-U 1722	N.E. El-Gholl	<i>Dodonea viscosa</i>	Florida, U.S.A.
	STE-U 1723	N.E. El-Gholl	<i>Nandina domestica</i>	Florida, U.S.A.
	ATCC 38227	S.A. Alfieri	<i>Mahonia bealei</i>	Florida, U.S.A.
	ATCC 46300	D.M. Benson	<i>Leucothoe catesbaei</i>	N. Carolina, U.S.A.
<i>Cy. pauciramosum</i>	STE-U 416	S. de Buisson	<i>Eucalyptus grandis</i>	N. Province, South Africa.
	STE-U 925	M.J. Wingfield	Soil	Santa Catarina, Brazil
	STE-U 972	P.W. Crous	<i>Eucalyptus grandis</i>	Western Cape, South Africa
<i>Cy. candelabrum</i>	STE-U 1677	A.C. Alfenas	<i>Eucalyptus</i> sp.	Amazonas, Brazil
	STE-U 1674	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil
	STE-U 1951	A.C. Alfenas	Soil	Brazil
<i>Cy. insulare</i>	STE-U 616	M.J. Wingfield	Soil	Amazonas, Brazil
	STE-U 768	P.W. Crous	Soil	Tamatave, Madagascar
	STE-U 954	M.J. Wingfield	Soil	Veracruz, Mexico
<i>Cy. mexicanum</i>	STE-U 927	M.J. Wingfield	Soil	Yucatan, Mexico
	STE-U 941	M.J. Wingfield	Soil	Campeche, Mexico
<i>Cy. ovatum</i>	UFV 90	A.C. Alfenas	Soil	Brazil
	STE-U 2232	P.W. Crous	<i>Eucalyptus</i> sp.	Brazil
<i>Cy. multiseptatum</i>	STE-U 1589	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia
	STE-U 1602	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia

Sexual compatibility

Isolates were mated in all possible combinations. This was achieved by removing 3 mm diam agar plugs from the periphery of actively growing cultures and placing them on carnation leaf agar plates as described by Crous et al (1997a). Two different isolates were placed in a Petri dish with carnation leaves between them. Plates were subsequently incubated for 2 mo at 22°C as explained in Part 2. Successful matings were regarded as those isolate combinations that produced perithecia with fertile, extruding ascospores. Perithecia were harvested and ascospores cultured on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa).

Isolation of DNA

Single conidial and ascospore isolates were grown on MEA plates and plugs transferred into 500 ml Erlenmeyer flasks containing 100 ml liquid MEA broth. Flasks were shaken at 25°C and 125 rpm for approximately 7 d. Mycelium was collected by filtration (Whatman no. 1 filter paper) and DNA was extracted as described by Crous et al (1993b).

RAPD analysis

PCR reactions (25 µl total volume) comprised of 1.5 units Biotaq (Bioline, London, U.K.) with the buffer as recommended by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM MgCl₂, 0.5 µM primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA. Reactions were performed on a Rapidcycler (Idaho Technology, Idaho, U.S.A.). RAPD reaction conditions consisted of the following: an initial denaturation for 30 s at 96°C, followed by 40 cycles of 30 s at 96°C, 30 s at 38°C and 30 s at 72°C. A final elongation step of 2 min at 72°C was included.

Amplified DNA fragments were separated on 1.6% (w/v) CE agarose gels (Boehringer Mannheim, South Africa), with ethidium bromide (1µg/ml) using 0.5 X TBE buffer and run at a constant voltage of 60 V. Fragments were visualised and photographed under ultraviolet light. Thirteen decameric oligonucleotides (OPE 02, 03, 04, 07, 09, 10, 11, 13, 15, 16, 17, OPM 06, OPY 20, Operon Technologies Inc., U.S.A.) were screened. One primer, OPE 17 (CTA CTG CCG T) was selected for further analysis after yielding polymorphic bands separating both parental isolates.

DNA fingerprints were evaluated by visual inspection of the photographs of the gels. Bands that were observed as intense bands were used for analysis. The data were scored on the presence or absence of fragments within each individual sample. Possible recombination observed in the parental isolates could be seen in progeny as determined by the co-segregation of bands that were polymorphic in the parents.

PCR amplifications

HMG box

The strategy of Arie et al (1997) was followed, using two degenerate primers based on the *Neurospora crassa* *mt a-1* HMG box, (NcHMG1 CCY CGY CCY CCY AAY GCN TAY AT and NcHMG2 CGN GGR TTR TAR CGR TAR TNR GG). DNA fragments were visualised on an agarose gel and photographed under ultraviolet light. Although several *Cylindrocladium* species were tested with the degenerate primers the clearest band was obtained from a homothallic species, *Cy. colhounii*. Fragments with an approximate size of 300 base pairs [based on known sequences from *Neurospora crassa* (Staben & Yanofsky 1990)] were subsequently cut from the gel with a clean scalpel. DNA was recovered from the agarose matrix using Wizard PCR Preps (Promega Corporation, Madison, Wisconsin). This was sequenced directly after purification. The amino acid translation from the sequence obtained was compared to the *N. crassa* *mt a-1* HMG sequence obtained from GenBank (M54787) (Staben & Yanofsky 1990) in order to confirm its identity. This sequence was used to design ColHMG1 (CCA GAT GCT GAA GCA GCT CAA CC) and ColHMG2 (GCT TCT TGA TGA GCT CAG CC). Fragments of approximately 170 base pairs were amplified and sequenced with these primers.

A range of different species in the genus *Cylindrocladium* from both mating types were tested for specific PCR of amplification of a *MAT-2* HMG box fragment using primers ColHMG1 and ColHMG2 under the following conditions: an initial denaturation for 2 min at 96°C, followed by 35 cycles of 15 s at 96°C, 30 s at 55°C and 35 s at 75°C. A final elongation step of 4 min at 75°C was included. PCR amplifications were performed on a Rapidcycler (Idaho Technology, Idaho, U.S.A.). Amplified DNA fragments were separated on 1.6% (w/v) CE agarose gels (Boehringer Mannheim, South Africa), with ethidium bromide (1 µg/ml) using 0.5 X TBE buffer and run at a constant voltage of 60 V.

β -tubulin gene

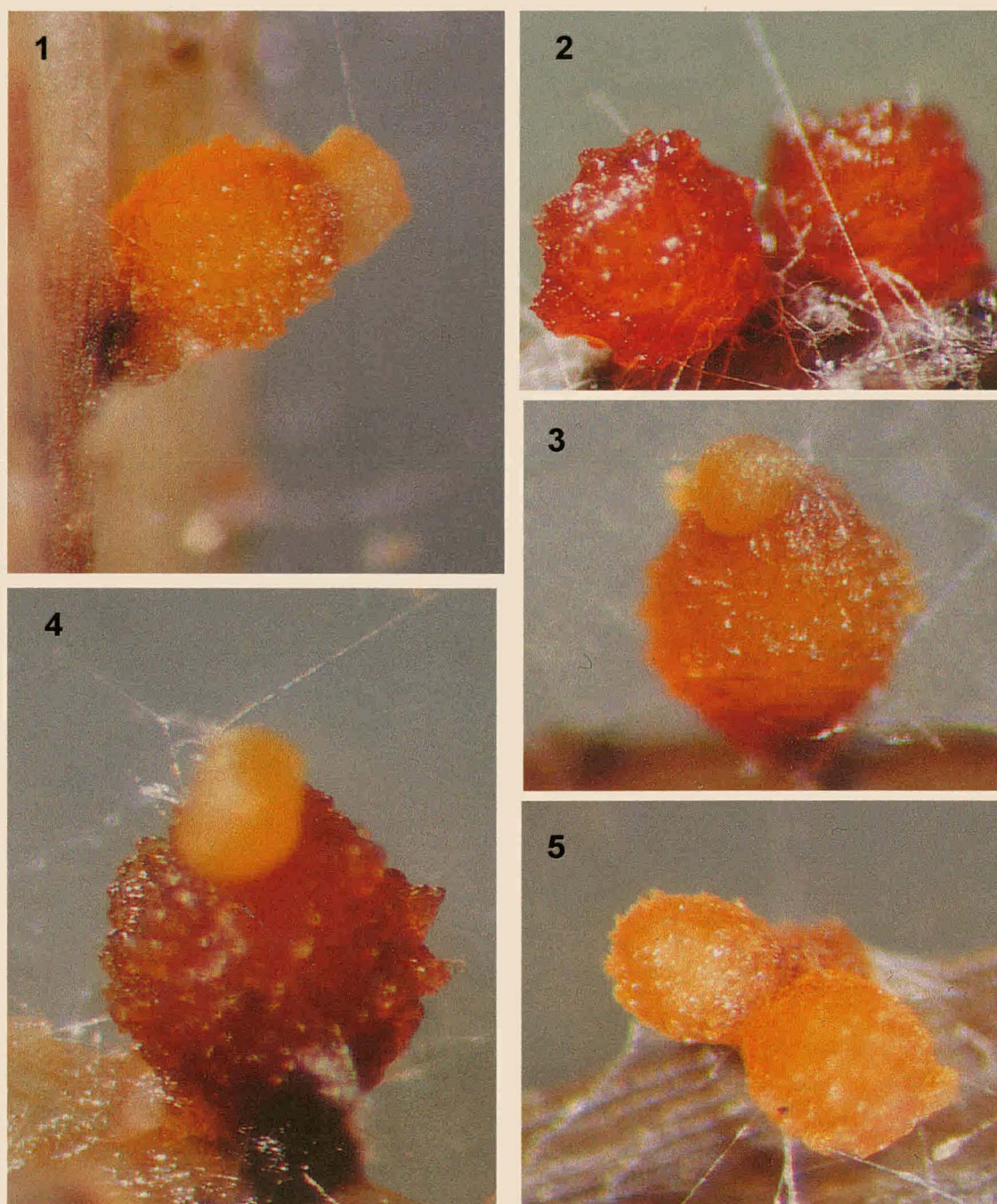
A 600 bp fragment was amplified with primers T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). Amplification and visualisation conditions were the same as for the HMG box.

ITS and 5.8S

The ITS1 and ITS2 internally transcribed spacers as well as the 5.8S ribosomal gene were amplified, yielding a fragment consisting of 537 bp. DNA was amplified using the primers ITS1 and ITS4 (White et al 1990). Amplification and visualisation conditions were the same as for the HMG box.

Sequence analysis

Initially both mating types of each species were tested for amplification with the primers ColHMG1 and ColHMG2. After the *MAT*-2 mating types were identified as those isolates yielding a fragment of approximately 300 bp, two isolates belonging to this mating type were used for further comparisons. Additional isolates belonging to the opposite mating type (based on the absence of the *MAT*-2 sequence) were used for the β -tubulin and ITS data sets. DNA was extracted as described by Crous et al (1993b) and PCR performed as mentioned previously. PCR products were purified using Wizard PCR Preps (Promega Corporation, Madison, Wisconsin). Both strands of the PCR product were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Connecticut, U.S.A.). Sequencing conditions were as described in Part 2. Alignments of sequences were done with the computer package Malign version 2.7 (Wheeler & Gladstein 1991) and appended manually. These were included in the Appendix (Alignments 1-3). Phylogenetic analysis of aligned DNA sequences was performed using PAUP* version 4.0b1 (Swofford 1998) and printed with the help of Treeview version 1.5 (Page 1996). Unweighted parsimony analysis was performed using the branch and bound search option. Gaps were treated as a fifth character, but in order to remove ambiguities only the first position was coded as such. Subsequent gap positions were coded as missing data. Confidence intervals for nodes were determined using 1000 bootstrap replications and the branch and bound search option for the β -tubulin and combined data sets. Due to the high number of possible trees the bootstrap values of the ITS data set was determined by means of a heuristic search with 1000 random additions and 1000 bootstrap replications.



Figs. 1-5. Variation in colour of perithecia from matings in *Cy. scoparium*. 1. Yellow perithecia from a cross of STE-U1720 X STE-U 1722. 2. Red immature perithecia from a cross between the two type species (ATCC 38227 X ATCC 46300). 3-5. Range of yellow to red perithecia from a cross of STE-U 1720 X ATCC 46300.

Results

Sexual compatibility

Crous et al (1993a) and Overmeyer et al (1996) previously reported that mating compatibility was low for *Cy. scoparium*. This was also true in the present study. From a total of ten isolates, including the reference isolates obtained from ATCC, only five isolates (STE-U 1720, STE-U 1722, STE-U 1723, ATCC 38227, ATCC 46300) could be crossed successfully. In the case of successful crosses fertile perithecia appeared after two to three wk. Successful crosses were: STE-U 1720 X STE-U 1722, STE-U 1720 X STE-U 1723, STE-U 1720 X ATCC 46300 and ATCC 38227 X ATCC 46300.

A successful mating between isolates STE-U 1720 and STE-U 1722 was selected for further study. Perithecia were found to be pale yellow to light orange in this cross (Figs. 1-5). Isolate STE-U 1720 also successfully mated with the reference isolate ATCC 46300 and this cross yielded perithecia ranging from pale yellow to orange brown in colour (Fig. 1-5). Viable progeny confirmed that these isolates belonged to the same biological species, namely *Cy. scoparium*.

After several unsuccessful attempts, a fertile cross could be observed between the two reference isolates (ATCC 46300 and ATCC 38227). Perithecial colour in this instance was as previously described, dark orange to red-brown (Figs. 1-5) (Crous et al 1993a, Overmeyer et al 1996). Ascospores were recovered from the mating between isolate STE-U 1720 and STE-U 1722. In addition to the fifteen ascospores used in th

RAPD analysis

The 15 randomly chosen ascospores were also used in the RAPD study. Primers were screened against the two parental isolates in order to find polymorphic bands between them. Most primers yielded profiles that appeared to be highly monomorphic. Only one primer showed clear polymorphic bands between the two parents, OPE 17 (Fig. 6). The markers shown in Fig. 6 co-segregated in three of the fifteen progeny (lanes 3, 7 and 17). Additional polymorphic bands in parent 1 (STE-U 1720) also co-segregated with the indicated polymorphic band (see arrow, Fig. 6) in parent 2 (STE-U 1722), further supporting the hypothesis that genetic material was derived from both parents. These data suggest that the ascospore progeny is the result of a true heterothallic cross and Mendelian segregation.

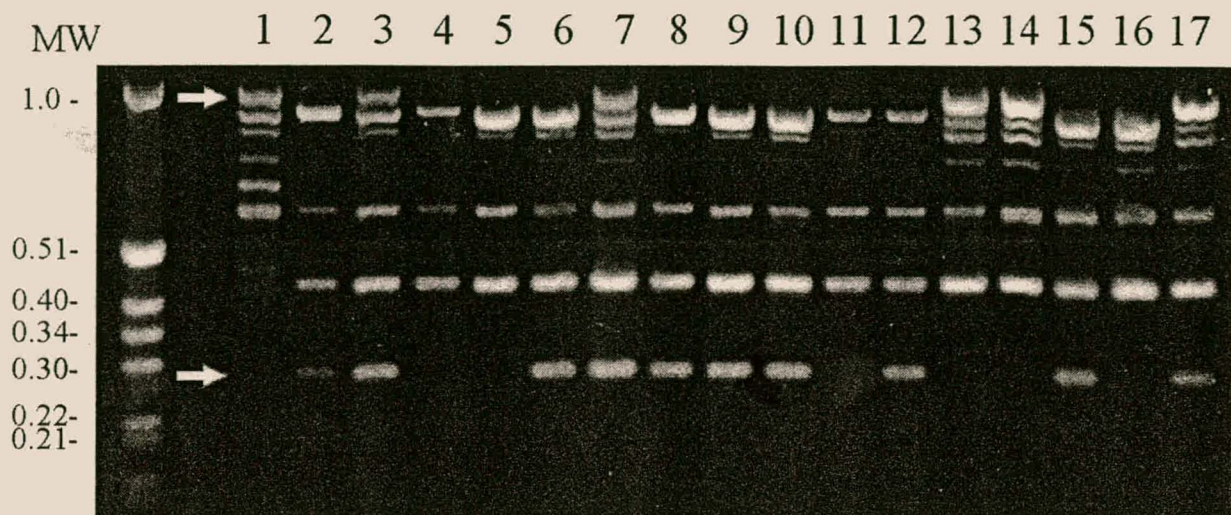


Fig. 6. Electropherogram showing RAPD profiles obtained with primer OPE 17. The two unique polymorphic bands are indicated (arrows). Lambda DNA size marker (kb) is also shown. Amplification products from parental isolates were loaded in lane 1 (STE-U 1720) and 2 (STE-U 1722). Products from ascospore progeny (A1-A15) are shown in lanes 3-17.

Phylogeny

Three regions of the genome were used for phylogenetic comparisons. The *Cylindrocladium* specific primers obtained from the *MAT-2* HMG box of an isolate of *Cy. colhounii* yielded products from several other *Cylindrocladium* species. Partial HMG box sequences from the *MAT-2* mating types of the small-spored heterothallic species, *Cy. scoparium*, *Cy. candelabrum*, *Cy. insulare*, *Cy. pauciramosum* and *Cy. ovatum* were also obtained. Where possible two isolates from disparate geographic areas were used for each species, in order to allow for intraspecific variation. In addition to this, the ITS ribosomal region and part of the β -tubulin gene were amplified and used for comparisons. Sequences of the opposite mating type for each species were also added to the β -tubulin and ITS data sets.

Two isolates from the multiseptate, large-spored species, *Cy. multiseptatum* Crous & M.J. Wingf., were included in order to investigate intrageneric phylogeny. The sequences of *Fusarium subglutinans* deposited by O'Donnell et al (1998), were obtained (GenBank accession numbers ITS: U34559, β -tubulin: U34417), and used as outgroups in the ITS and β -tubulin data sets. A sequence of the *Fusarium oxysporum* Shldl.:Fr. (O-17) obtained from Genbank (AB005040) was used as outgroup for the partial *MAT-2* HMG data set. The results were presented as phylogenetic trees (Figs. 7-9).

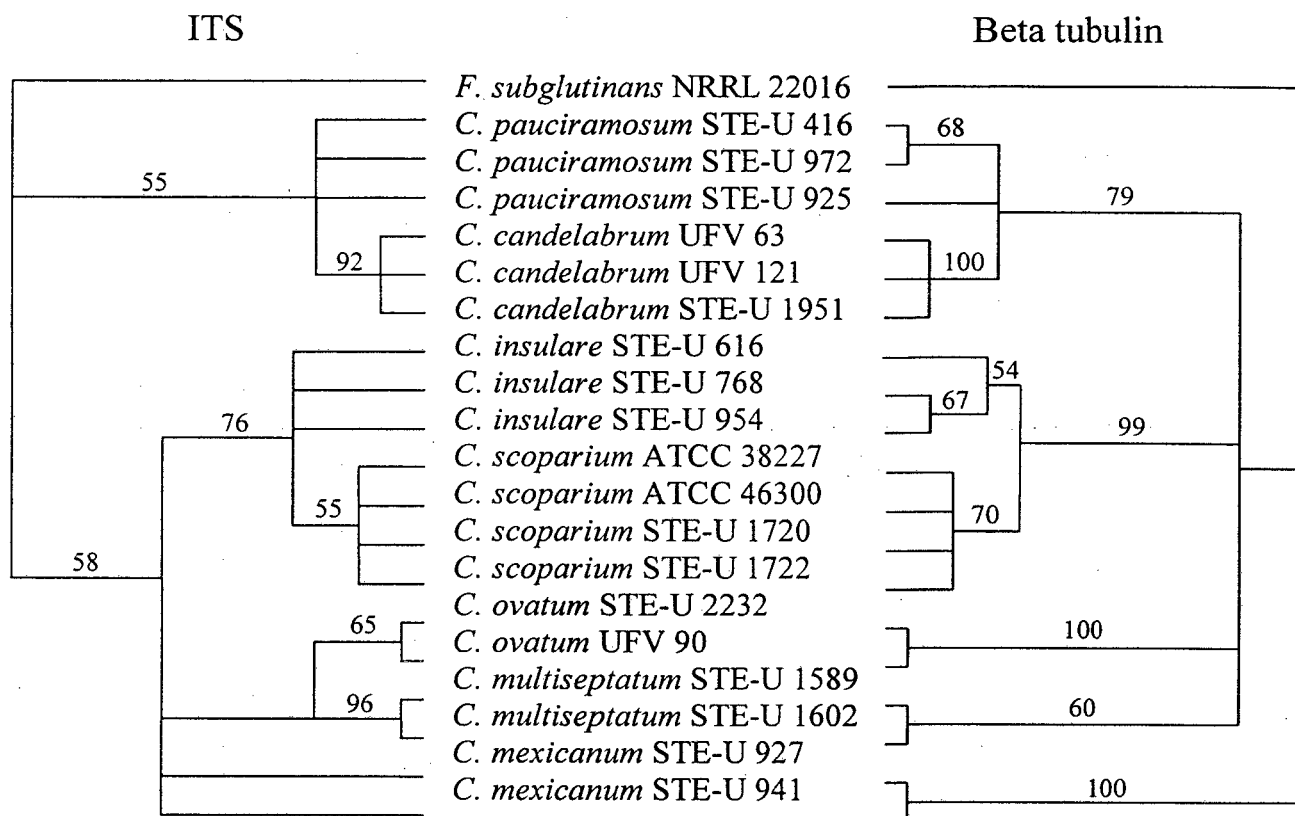


Fig. 7. Concordance of two selected most parsimonious trees generated from aligned sequences of the 5.8S gene and flanking ITS regions (done with a heuristic search with 1000 random addition sequences, 186 trees, 86 steps, CI=0.965, RI=0.941, RC=0.908) as well as the β -tubulin gene (with a branch and bound search, 27 trees, 320 steps CI=0.844, RI=0.854, RC=0.721) in PAUP* version 4.0b1. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown.

The ITS data set consisted of 489 nucleotide characters, of which 14 were parsimony informative, while the β -tubulin data set contained 107 parsimony informative sites out of 540 nucleotide characters. The area of the β -tubulin gene sequenced was found to have three introns containing 93 informative sites. Only 15% of informative sites were in the coding regions. Substitutions in the exons were favouring third base substitutions with 65% of all variable characters in this position, while 16% and 19% were in the first and second bases respectively.

Trees obtained from only the coding regions of the β -tubulin gene could not distinguish between the species *Cy. insulare* and *Cy. scoparium* as well as *Cy. pauciramosum* and *Cy. candelabrum* with any meaningful bootstrap support (results not shown), but were still concordant with a tree from the total β -tubulin data set (Fig. 7). A partition-homogeneity analysis performed on PAUP* version 4.0b1 revealed an underlying similarity in the phylogeny ($P = 0.84$) of the trees obtained with ITS and β -tubulin data sets. A similar analysis also indicated that the three introns in the β -

tubulin data set provided concordant phylogenies ($P=0.56$). The β -tubulin data set will be discussed in more detail in Part 5. The disparity in the number of informative characters in the ITS and β -tubulin data sets is reflected in the bootstrap values revealed in Fig. 7. Nodes generally had lower support in the ITS data set than in the β -tubulin data set. A closer relationship of the Brazilian isolate of *Cy. pauciramosum* (STE-U 925), with the apparent sibling species *Cy. candelabrum* is also evident from the β -tubulin data set. Both these taxa were shown to be biological species (Part 2), but a closer relationship between isolates from similar geographical origins is suggested from these data. This will be discussed in more detail in Part 4.

The topology for the tree based on the MAT-2 HMG box sequences (Fig. 8) confirmed the results discussed above. However, although isolates of *Cy. ovatum* were shown to be distinct from other isolates, relationships between this species and

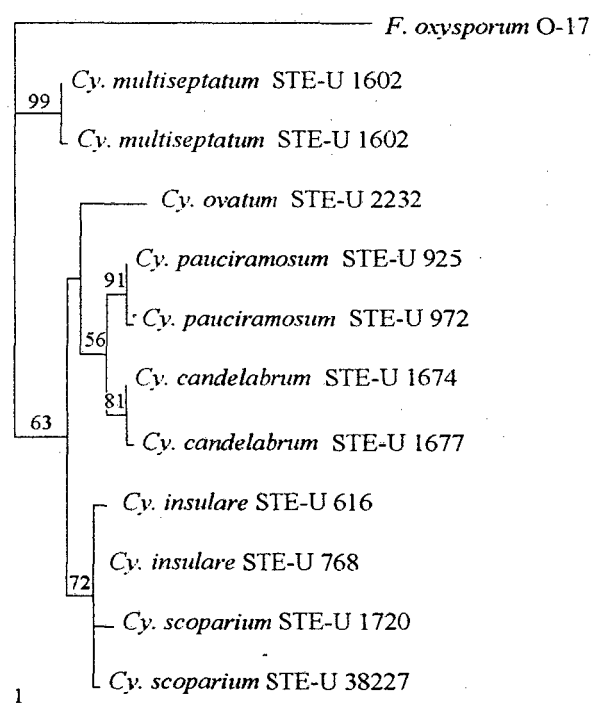


Fig. 8. One of 13 most parsimonious trees (48 steps CI = 0.917, RI = 0.920, RC = 0.843) generated by the branch and bound algorithm in PAUP* version 4.0b1 based on sequences of the MAT-2 HMG box. Clade stability was assessed with 1000 bootstrap replications (values above 50% are shown) and *F. oxysporum* was used as outgroup

other species were not concordant in all data sets. The use of *Fusarium subglutinans* and *Fusarium oxysporum* MAT-2 HMG box protein sequences obtained from GenBank (accession number AFO 25888) was used to confirm the identity of the sequences amplified. The high variation of the nucleotide sequences obtained for these *Fusarium* species made sequence alignment difficult. Therefore, sequences from the two isolates of *Cy. multiseptatum*, shown to group distantly in the ITS and β -tubulin data sets, were used as outgroup sequences. The MAT-2 HMG box data set consisted of 171 nucleotide characters, with 27 of these characters being parsimony

informative. In a similar fashion to the ITS data set, it was not possible to distinguish between isolates of *Cy. scoparium* and *Cy. insulare*. However, it was possible to separate both these species after analysis of the β -tubulin data, albeit with weak bootstrap support (50-70%).

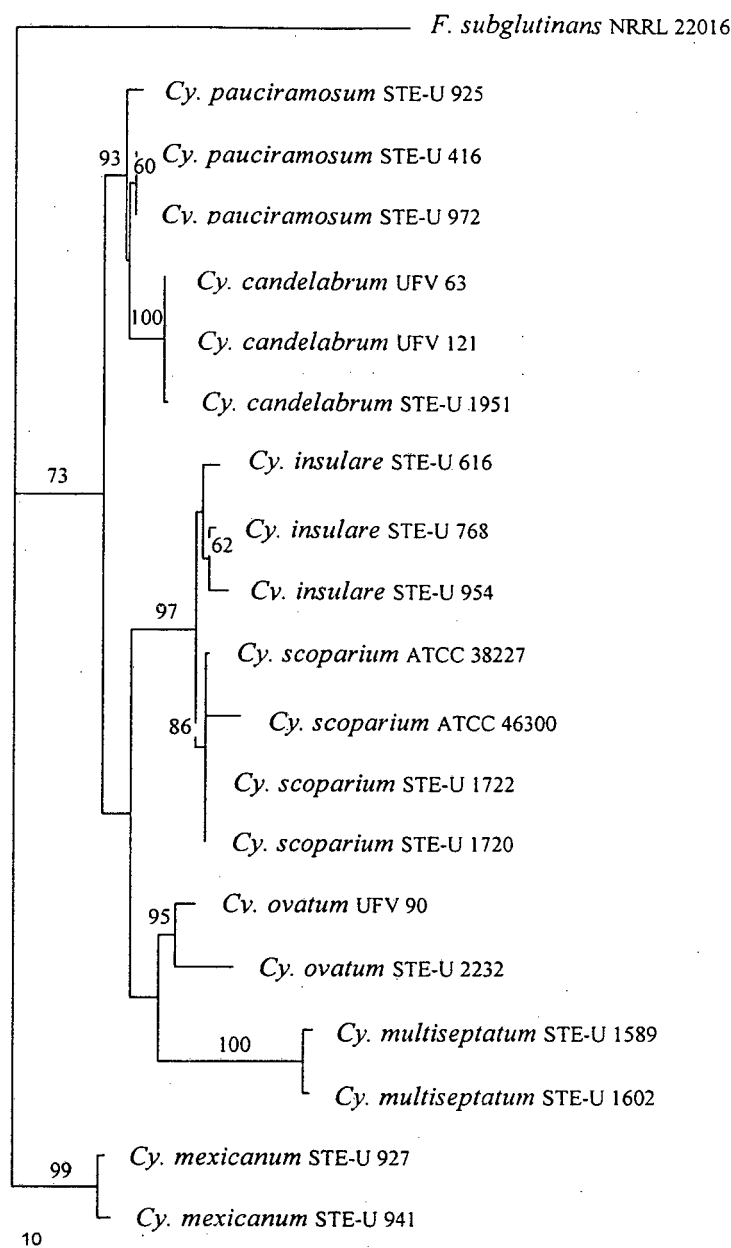


Fig. 9. Dendrogram of combined ITS and β -tubulin data set. One of 20 most parsimonious trees (531 steps CI = 0.857, RI = 0.824, RC = 0.706) generated with a branch and bound algorithm in PAUP* version 4.0b1 from aligned sequences of combined data set of the 5.8S gene and flanking ITS regions as well as the β -tubulin gene. Ten steps are indicated by the bar. Clade stability was assessed with 1000 bootstrap replications and values above 50 % are shown. *Fusarium subglutinans* sequences (GenBank-accession numbers ITS:U34559, β -tubulin:U34417) were used as outgroups.

A final analysis was done with a combination of both ITS and β -tubulin data sets (Fig. 9). The partition homogeneity test performed earlier indicated the possibility that these data sets would reinforce each other. This data set yielded 20 most parsimonious trees and consisted of 1026 nucleotide characters with 135 being parsimony informative. This confirmed the topology seen in the earlier dendrograms.

However higher bootstrap support for a separation of *Cy. scoparium* and *Cy. insulare* was observed. The high similarity previously mentioned in the RAPD data between STE-U 1720 and STE-U 1722 is also reflected.

Isolates of *Cy. multiseptatum* were shown to be distant from the other small-spored species in agreement with the difference in morphology. Isolates from *Cy. mexicanum*, the fourth species described under the *Cy. candelabrum* species complex, also grouped distantly compared to the other small-spored species. Neighbor-joining and maximum-likelihood trees for all data sets (results not shown) were concordant with those obtained through maximum parsimony.

Discussion

The results obtained in the present study have confirmed that *Cy. scoparium* has a biallelic heterothallic mating system. Furthermore, sequence data from all three genomic regions used also support *Cy. scoparium* as a morphological and biological species, distinct from other morphologically similar small-spored *Cylindrocladium* spp.

The results of the mating study are in direct contrast with those previously obtained (Overmeyer et al 1996), where a system involving genetic material from only one parent was suggested in *Cy. scoparium*. Using RAPD markers, recombinant profiles obtained from both the parental isolates (STE-U 1720 and STE-U 1722) were observed in the F1 generation. A phylogenetic analysis of RAPD data obtained by Overmeyer et al (1996), however, showed all progeny to group with one parent. In addition to this, no back-cross was reported with strain ATCC 38227. However, F1 isolates were reported to intercross, indicating the existence of both mating types in the sample used. The absence of protoperithecia reported by Overmeyer et al (1996), and observed in this study, indicate that isolate ATCC 38227 has lost the ability to act as a hermaphrodite in a cross. This fact, combined with the low fertility observed in our study could explain why Overmeyer et al (1996) were unsuccessful in backcrossing ascospore progeny with ATCC 38227.

Furthermore, RAPD results obtained from 15 ascospores in the present study indicate that both parents contributed to the genetic make-up of the progeny. The designation of all isolates as either *MAT-1* or *MAT-2*, using DNA sequence data, their

novel RAPD profiles as well as their mating behavior with tester strains, is further proof that a biallelic heterothallic system exists in *Cy. scoparium*.

In order to determine the phylogenetic relationships between other heterothallic, small-spored *Cylindrocladium* species and *Cy. scoparium*, several genomic DNA regions were sequenced and analyzed. This study evaluated the phylogenetic trees obtained from the *MAT-2* gene HMG box, β -tubulin and the ribosomal ITS region. From the results presented here it is clear that, in spite of their similar morphology, these species can be differentiated on the basis of DNA phylogeny. Although only an area of 170 base pairs was obtained from the HMG box, trees were similar in topology compared to those obtained from β -tubulin and ITS sequences.

The results further indicate that *Cy. scoparium* is very closely related to *Cy. insulare*. Only one area of the genome tested, β -tubulin, could distinguish isolates of these two species. This could not be done with high bootstrap support, however. In a combined data set of both ITS and β -tubulin sequences higher bootstrap values were observed (Fig. 9). A closer relationship between the two isolates selected for the mating studies (STE-U 1720 and 1722) is also evident with relatively high bootstrap support in the combined data set. This is in agreement with the high amount of monophyly observed with the RAPD markers. Additionally, the variation in perithecial colour observed between crosses of these isolates and those involving the ATCC reference isolates support this observation. This finding also underlines the fact that in some heterothallic species of *Calonectria* variation can occur regarding perithecial colour, thus reducing the usefulness of this feature for species identification (Crous & Wingfield 1994).

The β -tubulin based tree grouped isolate STE-U 925 of *Cy. pauciramosum* with isolates of *Cy. candelabrum*. All of these isolates were collected in Brazil. In other studies where β -tubulin sequences were obtained from a wider range of *Cy. pauciramosum* isolates (Part 4), clusters correlated with geographical origin, but also confirmed a close relationship among various South American species, and between *Cy. pauciramosum* and *Cy. candelabrum* in particular. The high similarity shown between these two species indicate that they probably are sibling species.

Other than rDNA ITS sequences, DNA sequences obtained from genes such as β -tubulin and *MAT-2* appear to be more variable and yielded much higher resolution for

interspecies differentiation. However, more information is needed regarding intraspecies variation and the relationship between some of the closely related species in *Cylindrocladium*, before these results can be seen as comprehensive.

Differing characters found for other *Cylindrocladium* species, such as optimum growth temperature (Crous & Wingfield 1994, Part 2), fungicide profiles (Jayasinghe & Wijesundera 1995) and pathogenicity (Alfieri et al 1972, Blum et al 1992, Crous et al 1993c) highlight the need for accurate identification of even seemingly closely related species. The fact that *Cy. scoparium* is regularly confused with morphologically similar species further underlines this requirement. This is exemplified by recent new reports of one of the species in the *Cy. candelabrum* species complex, *Cy. pauciramosum* from Italy (Polizzi & Crous 1999) and Florida (Koike et al 1999). The apparent lack of resolution in morphological characters in this genus necessitates the use of sexual compatibility (where applicable) as well as molecular characters in order to identify morphologically similar species.

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4. Comparison of female fertility and β -tubulin DNA sequences in isolates of *Cylindrocladium pauciramosum*

Abstract

Cylindrocladium pauciramosum isolates were obtained from nurseries in South Africa, Italy and the U.S.A. The percentages of hermaphrodites and the different mating types were evaluated in these isolates. This enabled the determination of the effective population for the different areas studied. All nurseries had isolates with mating type ratios significantly different from an expected 1:1 ratio. In the South African nursery, the *MAT-1* mating type was dominant, while the *MAT-2* was more common in other samples. This was consistent with one or more founder effects. The high percentage of hermaphrodites also suggested that recent introductions had occurred in nurseries in Italy and the U.S.A. DNA sequence comparisons of the 5' end of the β -tubulin gene obtained for a set of *Cy. pauciramosum* isolates collected from various geographic regions yielded different amounts of variation. All isolates from South Africa, U.S.A. and Australia had identical sequences. In the Italian sample, two groups were observed, one of which was identical to the sequences obtained from isolates in the other areas. Finally, a group of isolates obtained from South and Central America had the highest variation of all isolates investigated and also included isolates that had shared characters with another biological species, *Cy. candelabrum*.

Introduction

Cylindrocladium species are associated with *Calonectria* teleomorphs (Rossman 1979). Species are distinguished based on the morphological features of the anamorph, such as conidium, vesicle and phialide morphology, as well as cultural characteristics. Morphological features of the teleomorph tend to be more conserved and species identification based on these characters alone is generally not possible (Crous & Wingfield 1994).

Cylindrocladium candelabrum is a well-known root and leaf pathogen of numerous hosts. This species has regularly been confused with another species, *Cy. scoparium* Morgan (Doidge 1950, Botha & Crous 1992, Polizzi & Azzaro 1996). In

order to distinguish these two species, *Cy. scoparium* was delimited as having ellipsoidal to pyriform vesicles, while *Cy. candelabrum* was circumscribed as having ellipsoidal to obpyriform vesicles (Crous et al 1993). Mating studies have shown both these species to be distinct and heterothallic (Crous et al 1993, Part 2).

In Part 2 the existence of four genetically isolated mating populations within the boundaries of the existing morphological definition of *Cy. candelabrum* was demonstrated. DNA sequencing of the ribosomal ITS regions confirmed these to be separate entities and consequently four species were described. One of these species, described as *Cy. pauciramosum*, was described from isolates originating in Australia, Brazil, Colombia, Mexico and South Africa.

Published records indicate that *Cy. pauciramosum* has been associated with diseases of plants in South Africa for several years, but incorrectly referred to as *Cy. scoparium* (Doidge 1950, Darvas et al 1978, Lamprecht 1986, Botha & Crous 1992) and *Cy. candelabrum* (Crous et al 1993). Previous reports of a new disease attributed to *Cy. scoparium* from nurseries in Sicily, Italy (Polizzi & Azzaro 1996) were subsequently shown to be caused by *Cy. pauciramosum* (Polizzi & Crous 1999). In addition to this, another recent report confirmed the recent introduction of this fungus into Florida, U.S.A. (Koike et al 1999).

The phylogenetic relationship of *Cy. scoparium* to other heterothallic small-spored *Cylindrocladium* species was recently investigated by means of DNA sequence comparisons (Part 3). Although previous work on these fungi could distinguish closely related species based on small differences in the sequence of the 5.8S rDNA and flanking internal transcribed spacers (ITS1 and ITS2) (Jeng et al 1997, Part 3), the low number of informative characters made phylogenetic determinations difficult. The use of DNA sequences obtained from additional areas, such as the β -tubulin gene and the HMG box of the *MAT*-2 mating type gene yielded higher variation and could distinguish most species previously defined based on other characters (Part 2).

Cylindrocladium pauciramosum is self-sterile, and female structures consist of protoperithecia that can be spermatized by conidia or hyphae from opposite mating type isolates. A typical heterothallic ascomycete has been defined as a self-sterile hermaphrodite, capable of producing the female reproductive structures as well as male gametes (Leslie & Klein 1996). Generally these male functions can be performed by asexual spores, sexual spores or mycelia. Observations in *Gibberella*

fujikuroi have shown that the female function is lost regularly (Leslie 1995). These female sterile isolates can act only as males and were proposed to have a vegetative advantage during asexual reproduction because no resources would be required for the production of female reproductive structures (Leslie & Klein 1996). The opposite scenario was proposed for conditions favouring sex, resulting in a higher percentage of hermaphrodites (Leslie & Klein 1996). The ratios of both mating types and of female steriles and hermaphrodites can be used to determine the importance of sexual replication and the effective population (N_e), giving an estimate of a finite population's size as first proposed by Wright (1931). These principles were reviewed by Caballero (1994) and adapted for haploids (Leslie & Klein 1996). Recent work by Mansuetus et al (1997) and Britz et al (1998) made use of these assumptions in order to gain information on the effective population size and sexual dynamics of mating populations in the *Gibberella fujikuroi* complex.

The goals of this study were to firstly determine the ratios of both mating types in the newly introduced populations of *Cy. pauciramosum* in Sicily (Polizzi & Azzaro 1996) and California (Koike et al 1999) and to compare these with a sample of the South African population. Additionally, the presumed founder populations introduced into disease free nurseries are compared with respect to fertility and mating type ratio. This would provide the necessary data to determine the mating type and inbreeding effective populations in the nurseries sampled. A final aim was to obtain data relating to infraspecific variation of *Cy. pauciramosum* based on DNA sequences of the β -tubulin gene of isolates collected from a wide geographical area.

Materials and Methods

Isolates

Isolates of *Cy. pauciramosum* (Table I) were either obtained from symptomatic plant material, or baited from soil samples. Soil samples were collected and treated according to Crous et al (1997). Collectors are indicated in Table I. All isolates were identified using the morphological concepts, mating types and keys as defined in earlier studies (Crous et al 1997, Part 3). For the purpose of this study mating capability of isolates was assumed not to be influenced by the host from which they were isolated, because species in the genus have been found not to be host specific and are essentially soil borne (Part 6).

South African isolates of *Cy. pauciramosum* were obtained from the culture collection at the Department of Plant Pathology at the University of Stellenbosch (STE-U). These were collected throughout South Africa over a period from 1990-1995 and were obtained from diseased plant material as well as from soil. Because a recent subset from this collection all produced successful crosses, it was assumed that the techniques used to preserve cultures did not adversely affect mating ability (Part 2). An additional sample of 50 isolates was obtained from crown and root rot symptoms on cherry, *Prunus* sp., plants (one isolate / plant) from a small nursery in Stellenbosch to which this disease was recently introduced (C. Linde pers. comm.).

Italian isolates were obtained from a number of nurseries in Italy (Polizzi & Crous 1999). A total count of 50 isolates was spread between several nurseries. In a similar manner, 50 isolates were collected from crown and root rot symptoms of heath, *Erica capensis* Salter from a single nursery in California, U.S.A.

Sexual compatibility

Two mating tester strains of the opposing mating type (*MAT-1* = STE-U 416, *MAT-2* = STE-971) were selected for their high levels of fertility during previous mating experiments (Parts 2 and 3). Single isolates were grown on Petri dishes containing malt extract agar (MEA) (Biolab, Midrand, South Africa) for 2-4 wk until sporulation. One ml of sterile water was added to each Petri dish and conidia were dislodged with the help of a sterile glass rod. The conidial suspension was removed with a micropipette. Cultures were spermatized by applying the conidial suspension to Petri dishes containing CLA with 2-4-wk-old growth. The selected cultures were spermatized with both tester strains. In addition to this, the tester strains were individually spermatized with all test isolates. Plates were packed in stacks of 10, sealed in plastic bags and incubated on the laboratory bench at 22°C. Successful crosses were determined after 2 mo of incubation and were selected as those isolate combinations that produced perithecia with extruding, fertile ascospores.

Statistical analysis

The effective population numbers were calculated according to methods of Leslie and Klein (1996). The effective population number based on mating type ($N_{e(mt)}$) was determined as $N_{e(mt)} = (4N_{MAT-1}N_{MAT-2}) / (N_{MAT-1} + N_{MAT-2})$ with N_{MAT-1} the number of *MAT-1* strains and N_{MAT-2} the number of *MAT-2* mating type strains. These are parameters to estimate genetic drift and inbreeding in populations. The inbreeding effective

population ($N_{e(t)}$) is based on the probability of identity due to common ancestry and determined as $N_{e(t)} = (4 N^2 N_h)/(N + N_h)^2$ with N being the total number of individuals and N_h the total number of hermaphrodites.

Isolation of DNA

Single conidial isolates selected for DNA comparison (Table II) were grown on MEA plates. Mycelial mats were removed from the plates by means of a sterile scalpel and ground to a powder by means of liquid nitrogen and a mortar and pestle. Approximately 40 mg of ground mycelium was added to 2 ml microtubes containing 600 μ l of extraction buffer. The extraction buffer consisted of 1% SDS, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 100 mM EDTA. Subsequently, the protocol was followed as suggested for the Wizard Genomic DNA Purification kit (Promega, Madison, U.S.A.).

PCR amplifications and sequencing

Reactions (total volume 25 μ l) comprised of 1.5 units Biotaq (Bioline, London, U.K.) with the buffer as supplied by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM $MgCl_2$, 0.5 μ M primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA as target. Reactions were performed on a Rapidcycler (Idaho Technology Idaho, U.S.A.). Reaction conditions consisted of the following: an initial denaturation for 2 min at 96°C, followed by 30 cycles of 15 s at 96°C, 30 s at 55°C and 35 s at 75°C with a slope of 1.0. A last elongation step of 2 min at 75°C was included. A 600 bp fragment was amplified using primers T1 (O' Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). PCR fragments were sequenced as described previously (Part 2).

Phylogenetic analysis

Sequence comparisons based on DNA sequences of the β -tubulin gene have previously been used to investigate phylogeny in *Cy. scoparium*, *Cy. pauciramosum* and related species (Part 3). In the present study an investigation on the variation in *Cy. pauciramosum* was undertaken. *Cy. candelabrum*, *Cy. multiseptatum* and *Fusarium subglutinans* were used as outgroups. As far as possible six isolates from disparate regions within a country and representing different mating types were used for comparison. The isolates selected for phylogenetic analysis are shown in Table II. Alignments of sequences were done with the computer package Malign version 2.7 (Wheeler & Gladstein 1991) and assessed manually. These are included in the

Appendix (Alignment 2). Phylogenetic analysis of aligned DNA sequences was performed using PAUP* Version 4.0b1 (Swofford 1998). Gaps were treated as a fifth base. Confidence intervals were determined using a 1000 bootstrap replications.

Table II. Isolates of *Cy. pauciramosum* and other species used for sequencing.

Species	Original no.	Collector	Host	Origin
<i>Cy. pauciramosum</i>	STE-U 143	P. W. Crous	<i>Eucalyptus grandis</i>	Mpumalanga, South Africa
	STE-U 416	S. de Buisson	<i>Eucalyptus grandis</i>	Northern Province, South Africa
	STE-U 344	P.W. Crous	<i>Eucalyptus grandis</i>	KwaZulu Natal, South Africa
	STE-U 925	M.J. Wingfield	Soil	Santa Catarina, Brazil
	STE-U 951	M.J. Wingfield	Soil	Veracruz, Mexico
	STE-U 971	P.W. Crous	<i>Eucalyptus grandis</i>	Western Cape, South Africa
	STE-U 972	P.W. Crous	<i>Eucalyptus grandis</i>	Western Cape, South Africa
	STE-U 1160	M.J. Wingfield	Soil	Córdoba, Colombia
	STE-U 1670	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil
	STE-U 1671	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil
	STE-U 1691	D. Hutton	<i>Fragaria</i> sp.	Queensland, Australia
	STE-U 1692	D. Hutton	<i>Fragaria</i> sp.	Queensland, Australia
	STE-U 1990	S. Koike	<i>Erica</i> sp.	California, U.S.A.
	STE-U 2030	S. Koike	<i>Erica</i> sp.	California, U.S.A.
	DISTEF-G 2	G. Polizzi	<i>Polygala myrtifolia</i>	Catania, Sicily, Italy
	DISTEF-G 6	G. Polizzi	<i>Callistemon citrinus</i>	Messina, Sicily, Italy
	DISTEF-G 60	G. Polizzi	<i>Myrtus communis</i>	Catania, Sicily, Italy
	DISTEF-G 62	G. Polizzi	<i>Callistemon citrinus</i>	Messina, Sicily, Italy
	DISTEF-G 84	G. Polizzi	<i>Acacia retinodes</i>	Messina, Sicily, Italy
	DISTEF-G 126	G. Polizzi	<i>Arbutus unedo</i>	Catania, Sicily, Italy
<i>Cy. candelabrum</i>	STE-U 1677	A.C. Alfenas	<i>Eucalyptus</i> sp.	Amazonas, Brazil
	STE-U 1674	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil
	STE-U 1951	A.C. Alfenas	Soil	Brazil
<i>Cy. mexicanum</i>	UFV 89	A.C. Alfenas	Soil	Brazil
	STE-U 927	M.J. Wingfield	Soil	Yucatan, Mexico
	STE-U 941	M.J. Wingfield	Soil	Campeche, Mexico
<i>Cy. multiseptatum</i>	STE-U 1589	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia
	STE-U 1602	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia

Results

Sexual compatibility

Effective populations and ratios based on numbers of mating types and hermaphrodites of the *Cy. pauciramosum* samples obtained from the selected areas are shown in Table III. These values reflect differences in the profiles for the different nurseries. Samples from a group of various areas in South Africa, where the disease has been well established, tended to have a mating type ratio of approximately 1:1. All other samples, representing areas where the pathogen is thought to be recently introduced, yielded ratios that significantly favoured one mating type. In the Stellenbosch nursery the ratio favoured the *MAT-1* mating type, while the nurseries in California and Sicily had more *MAT-2* isolates present. Additionally, only one mating type, *MAT-2*, was present in Californian nursery. These figures differ appreciably

from those obtained by other workers for species of the *Gibberella fujikuroi* complex (Leslie & Klein 1996, Mansuetus et al 1997, Britz et al 1998). Here the highest mating type ratio was approximately of 1:2.

Table III. Comparison of population distribution of mating types and hermaphrodites between three geographic areas.

Geographic origin	Ratio of mating types ¹	N_e (effective population) ³		
		$N_{fs}:N_h$ ²	$N_{e(mt)}$ ⁴	$N_{e(f)}$ ⁵
South Africa				
Stellenbosch nursery	48:8	31:25	49.0	85.3
Rest of South Africa	21:23	29:15	99.7	75.8
United States				
Californian nursery	0:50	4:46	0	99.8
Italy				
Various nurseries	13:41	12:42	73.1	98.4

¹ The ratio of mating types based shown as MAT-1:MAT-2 (mating types determined previously according to work done in Part 3).

² The ratio of female sterile:hermaphrodites in the population.

³ The effective population number based on the numbers of males (N_{fs}) and hermaphrodites (N_h) as percentage of the actual count.

⁴ Effective population number based on mating type (given as percentage of total population).

⁵ Inbreeding effective number based on numbers of males and hermaphrodites (given as percentage of total population).

Equations were all derived from Leslie and Klein (1996).

Effective population size, based on mating types ($N_{e(mt)}$), of between 49% and 99% of the total count was inferred where both mating types of the *Cy. pauciramosum* isolates were present. In contrast to the effective populations based on mating types, higher effective populations based on the presence of hermaphrodites were found. Other effective population values differed between 76% and 98% of the total population. High percentages were found for nurseries in California and Italy (98-99%), in spite of a mating type bias in these samples. Mating type ratios found for the hermaphrodites were also comparable to those of the total sample in all cases (data not shown).

β -tubulin sequence analysis

Based on preliminary results obtained in Part 3 a number of isolates were selected in order to investigate the variation of the β -tubulin DNA sequence for a number of isolates within *Cy. pauciramosum*. A heuristic search using PAUP* 4.0b1 with 500 random additions and 1000 bootstrap repetitions yielded ten most parsimonious trees. One of these trees is shown in Fig. 1.

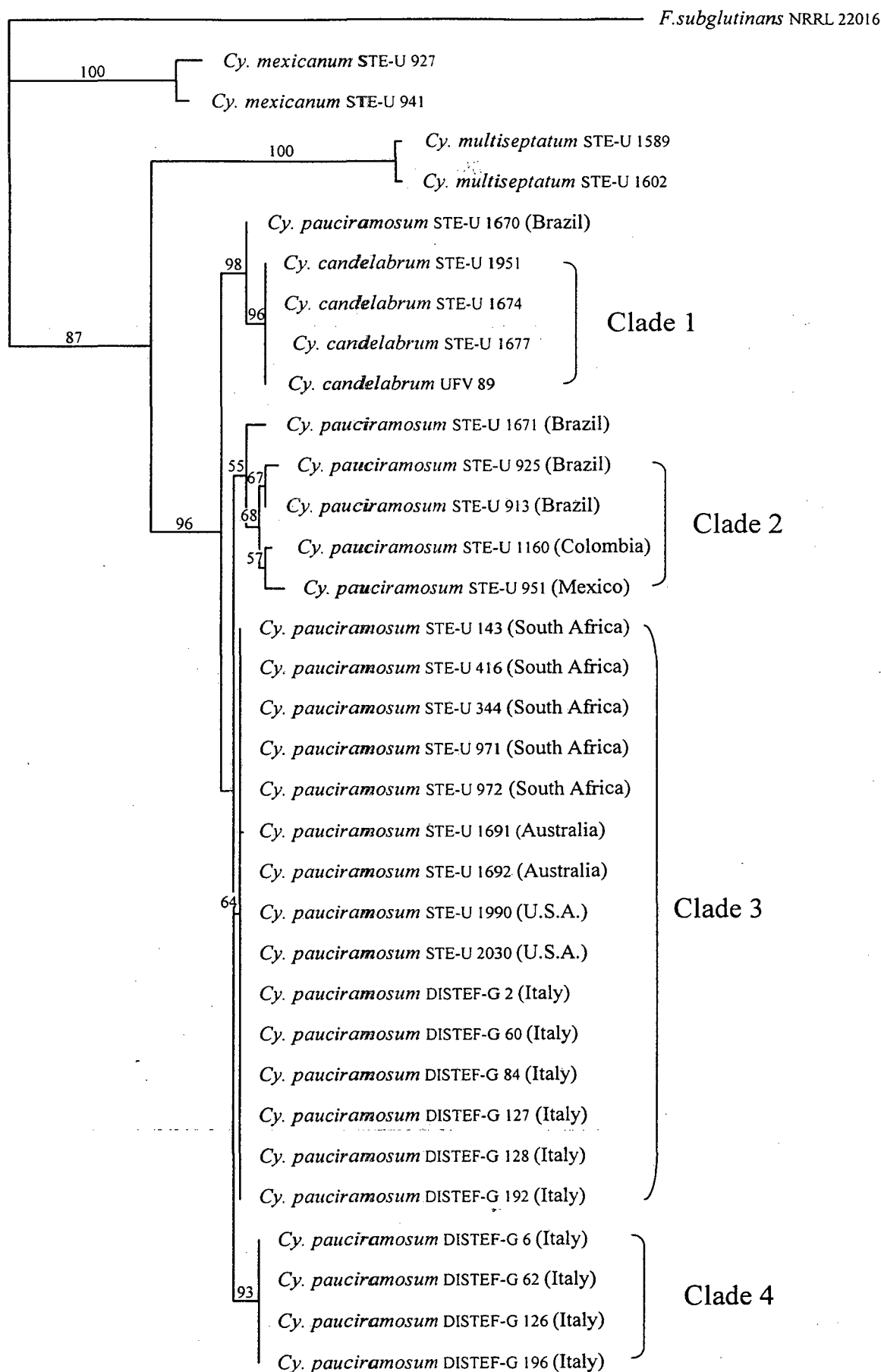


Fig. 1. Phylogram of DNA sequences from the 5' end of the β -tubulin gene. One of 10 most parsimonious trees (266 steps, CI=0.925 RI=0.905 RC=0.837) generated by the heuristic algorithm in PAUP* version 4 based on sequences of the β -tubulin gene. Ten steps are indicated by the bar. Clade stability is assessed by 1000 bootstrap samplings (shown above branches).

All variable characters in the DNA alignments were single base pair substitutions, less than a third of these being transversions. From a comparison between *Cy. candelabrum* and *Cy. pauciramosum* 27 variable characters were found out of a data set of 521 unordered characters. Only 18 of these were parsimony informative. This is comparable to the low, but consistent amount of variation previously seen between species of *Cylindrocladium* in the ITS1 and ITS2 spacers flanking the 5.8S ribosomal RNA gene (Crous et al 1999, Part 2). Variable characters were almost exclusively situated in the non-coding regions of the β -tubulin gene. Only 2 characters (base pairs 273 and 348) were inside the coding regions (Table IV).

The low number of informative characters were emphasised in the low bootstrap values (Fig. 1). A Neighbor Joining comparison done with 1000 bootstrap replications in PAUP* yielded higher values (75-90%) and similar topology. In order to investigate the variation at sequence level the different base pair substitutions are presented as single characters in Table IV with the clades as indicated in Fig. 1.

Cylindrocladium candelabrum isolates obtained from Brazil (Clade 1, Fig. 1) all had identical sequences. Although this species is closely related to *Cy. pauciramosum*, these two species have already been shown to differ biologically and genetically (Parts 2 and 3). The *Cy. candelabrum* isolates (Clade 1) shared a total of nine variable sites of which three (base pairs 57, 232 and 409) were unique for all isolates in this group. The remaining five variable sites were shared by isolates in clade 2 with an isolate of *Cy. pauciramosum*, STE-U 1670. This isolate clustered with *Cy. candelabrum* (Fig. 1), but still grouped separate and also had one unique variable site (base pair 227). Only one variable site separated clades 1, 2 and STE-U 1670 (base pair 198).

Variation was found for other individual *Cy. pauciramosum* isolates from South America and Mexico. Three variable characters were found in Clade 2. Of these, base pair 198 was shared with clade 1 and STE-U 1670, and base pair 420 with STE-U 1671. Although variation occurred between South American and Mexican isolates no variable characters were shared with any of the isolates from the other geographic regions (South Africa, Australia and Italy).

Isolates in Clade 3, selected from the South African, Italian and California populations had identical sequences and clustered together with low bootstrap support. This group is supported by one unique character at base pair 95 (Table IV).

A different group of Italian isolates were supported by four unique base pair substitutions (Clade 4). This grouping is shown to be distinct with high (93%) bootstrap support (Fig. 1).

Table IV. The 27 variable characters in the comparison of β -tubulin DNA sequence data from isolates of *Cy. pauciramosum* *Cy. candelabrum* species, compared to the groups seen in Fig. 1, and their areas of origin. Base pairs are numbered from the start of deposited sequences (Appendix, Alignment 4).

Base pair no.	Original state	Derived state	Group (seen in Fig. 1)	Geographic origins
57	C	T	Clade 1	Brazil
63	C	G	STE-U 925	Brazil
75	A	G	STE-U 1671	Brazil
83	C	G	STE-U 925	Brazil
95	A	G	Clade 3	Australia, California, South Africa, Italy
138	C	T	Clade 1, STE-U 1670	Brazil
143	T	C	STE-U 951	Mexico
181	T	C	Clade 1, STE-U 1670	Brazil
195	T	C	Clade 1, STE-U 1670	Brazil
198	A	C	Clade 1, STE-U 1670, Clade 2	Brazil
212	G	A	STE-U 951, STE-U 1160	Mexico, Colombia
215	A	G	STE-U 913, STE-U 925	Brazil
217	T	G	STE-U 1671	Brazil
219	A	G	Clade 2	Brazil
225	A	G	STE-U 951	Mexico
227	T	C	STE-U 1670	Brazil
232	G	C	Clade 1	Brazil
273	T	C	Clade 1, STE-U 1670	Brazil
348	C	T	Clade 4	Italy
387	C	G	Clade 4	Italy
394	C	T	Clade 4	Italy
397	G	A	STE-U 1160	Colombia
406	G	T	Clade 1, STE-U 1670	Brazil
409	A	G	Clade 1	Brazil
413	C	G	Clade 4	Italy
417	C	T	STE-U 951	Mexico
420	C	A	Clade 2, STE-U 1671	Brazil

Discussion

The results presented here showed fundamental differences in the profiles of the populations sampled. In effect, all the nursery populations amounted to founder populations and some could have gone through several population bottlenecks. This is reflected in the varying ratios of mating types found in the different nurseries in the different geographic areas. The only population that approached a 1:1 mating type ratio was the sample where the disease has been well established in South Africa (Table III). This was collected over a wide area and a time period of several years.

The Italian sample used in the present study resembled a number of isolates spread over a number of nurseries in Sicily and Southern Italy. Preliminary results obtained from an additional number of isolates collected in single nurseries such as Carubba,

Barcelona and Milazzo indicate that the founder effects seen in the nurseries of Stellenbosch (South Africa) and California (U.S.A.) were also consistent in these circumstances (results not shown). Indications are that in some Italian nurseries only one mating type has been introduced. All the Italian samples had the same mating type bias correlating with a single source, the nursery in Carubba. This nursery has been established as the point of entry for new material in the region and could have had a persistent inoculum (Sicily and Southern Italy). Further analysis of these additional samples will enable a clearer picture of the population variation between single nurseries in Italy.

The high ratios of hermaphrodites in samples supports hypothesised recent introductions (Polizzi & Azzaro 1996, Koike et al 1999). However, the percentage of hermaphrodites found in the various nurseries is still consistent with a population that is sexually reproductive (Leslie & Klein 1996, Britz et al 1998). One would expect the percentage of hermaphrodites to drop if a single mating type were to persist in each nursery. The application of good nursery practices entails the immediate removal of diseased material. This has the potential to create several bottlenecks as the remaining populations must result from small starter populations. The influx of new diseased material containing the opposite mating type could further rapidly influence population structure in these nurseries.

Plant pathogens are normally introduced into nurseries by infected plant material or soil. The most important survival structures of *Cylindrocladium* spp. are microsclerotia which can survive for periods of up to 15 years and longer in soil (Thies & Patton 1970, Sobers & Litrell 1974). Under suitable climatic conditions germination and subsequent infection of roots and leaves occurs (Anderson et al 1962, Sharma et al 1990). The conidia form on infected plant material and are splash dispersed between closely placed plants (Mohanani & Sharma 1986). In the case of sexual reproduction, the ascospores can also be an additional source of inoculum and are generally wind dispersed (Crous et al 1991). The profiles of the mating type distributions found in this study are consistent with the effects seen for a small initial inoculum, probably by asexual propagules. The fact that only one mating type can be found in the nursery samples from California, as well as the strong bias towards one mating type, suggests that sexual replication has a small role to play under these circumstances.

Genetic variation, based on DNA sequencing data, was detected between different isolates of *Cy. pauciramosum*. Although the gene phylogeny as reflected from the tree obtained from the partial sequences of the β -tubulin gene may not accurately reflect the species phylogeny (Doyle 1992, Maddison 1997), recent analysis of different loci have produced concordant phylogenies for *Cy. pauciramosum* and closely related species (Part 3).

Shared characters were found between a number of *Cy. pauciramosum* isolates and isolates of *Cy. candelabrum*. These *Cy. pauciramosum* isolates include the isolates in Clade 2 as well as STE-U 1670 (Fig. 1). All of these isolates were collected in South and Central America. This suggests a population of *Cy. candelabrum* being sexually isolated from the more variable mother population of *Cy. pauciramosum*. In addition to this, it would imply that these two taxa are sibling species.

The high variation amongst South and Central American isolates of *Cy. pauciramosum* is consistent with an endemic population in this area. Attempts to obtain a larger sample to include in this study have thus far proved unsuccessful as all samplings contained mainly *Cy. candelabrum* isolates. These results could imply a South African population introduced from elsewhere. The identical DNA sequences obtained from the South African isolates certainly allows this possibility. DNA sequences obtained from isolates collected from a wide variety of locations, including Australia, South Africa, Italy and California were also identical and could indicate a collective origin for these populations. There is anecdotal evidence of importation of South African nursery material into Italian nurseries and this would agree with results presented here. The occurrence of another distinct group of DNA sequences obtained from isolates in the Italian population complicates this issue. It is possible that there has been more than one introduction of this species into this area.

Because the relatively small sample sizes utilised in this study could influence the results, it must be emphasised that this is a first approximation of the variation present in populations of *Cy. pauciramosum*. A more detailed study of genetic and mating markers will allow more comprehensive conclusions to be drawn. In spite of this, these results emphasise the importance of identification of the members of morphologically closely related species in the *Cylindrocladium candelabrum* species complex in order to aid phytosanitation programmes and aid disease control.

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5. Phylogeny of *Calonectria* based on β -tubulin DNA sequence comparisons

Abstract

The phylogeny of the genus *Calonectria* was analysed by means of DNA sequence comparisons. This was done by amplifying the 5' end of the β -tubulin gene from isolates representative of 30 *Calonectria* species. A neighbor-joining analysis was performed on a total data set of 86 isolates, while a representative subset of isolates was analysed by means of maximum parsimony. The analyses yielded dendrograms with concordant topology. Many clades, containing small numbers of isolates were strongly supported by bootstrap. However, relationships between these clades were often ambiguous. A number of phylogenetic placements based on DNA data did not always agree with preconceived morphological relationships. Two large groupings were evident and both contained small-spored one-septate species. The only morphological character that correlated with DNA based phylogenies was vesicle shape.

Introduction

The genus *Calonectria* De Not. resides in the euascomycete order Hypocreales and has been characterised as having *Cylindrocladium* Morgan anamorphs (Rossman 1979, Crous & Wingfield 1994). Members of this genus are defined as species with brightly coloured ascocarps that change colour when placed in a 3% KOH solution (KOH+), have a warty wall surface, darkened stromatic bases, as well as *Cylindrocladium* anamorphs (Rossman 1993, Rossman et al 1999). *Cylindrocladium* anamorphs are the form most frequently encountered in nature and are also morphologically the most informative. Thus, most of the species in *Calonectria* are distinguished based on morphological features of their anamorphs.

Conflicting opinions have arisen regarding the use of the stipes emanating from the conidiophores and specifically the shape of its terminal vesicle as a taxonomic character. This character was rejected by some authors (Hunter & Barnett 1978, Rossman 1983), but others found it to give reliable taxonomic results (Sobers & Alfieri 1972, Peerally 1991). Crous et al (1992) demonstrated that the osmotic potential of the medium influences vesicle shape. Thus vesicle shape was proposed to be a reliable character only when it is used under standardised conditions.

Consequently, this criterion has been combined with other morphological characters in order to delimit *Cylindrocladium* species (Crous & Wingfield 1994).

Several *Cylindrocladium* species have been described with variable morphological characters despite the use of standardised growth conditions. One such character is conidial septation. Hence only predominant septation has been used as an important character in past descriptions (Crous & Wingfield 1994). Further studies have also shown intraspecific variation in other characters such as conidial size and vesicle shape (Crous & Peerally 1996, Crous et al 1998).

Because various morphological characters overlap in *Cylindrocladium*, frequent misidentifications occur, and different biological species are commonly lumped together under broad morphological species concepts. One example is the *Cy. candelabrum* Viégas species complex. Besides the fact that it is regularly confused with other species such as *Cy. floridanum* Sobers & C.P. Szym., *Cy. ovatum* El-Gholl et al and *Cy. scoparium* Morgan, a high amount of plasticity within the limits originally defined for its vesicle shape has been reported (Crous et al 1993a). A subsequent study revealed the presence of several distinct mating populations previously identified as *Cy. candelabrum* (Part 2). The use of mating tester strains was advocated in order to differentiate between these biological species, but due to the practical limitations of this approach, molecular characters became increasingly significant. In agreement with the contemporary trend in systematics, such molecular characters have also been applied to *Calonectria* taxonomy. These include the use of aminopeptidase substrate specificities (Stevens et al 1990), total protein electrophoresis (Crous et al 1993a) isoenzyme comparisons (El-Gholl et al 1997), DNA hybridisation based techniques (Crous et al 1993b, Victor et al 1997) as well as PCR-based methods (Victor et al 1997). These techniques have been helpful in delimiting several new species (Crous et al 1997a, Victor et al 1997, Crous et al 1999) subsequent to the monograph by Crous and Wingfield (1994).

The first study using DNA sequence comparisons to distinguish species of *Cylindrocladium* was that of Jeng et al (1997), where isolates of *Cy. floridanum* were compared with *Cy. scoparium* using the DNA sequences obtained from the ITS-5.8S ribosomal RNA area. Although these authors found differences between the two species, subsequent work showed that these differences were consistent between other *Cylindrocladium* species, and that the number of variable characters in this

region was low (Part 2). This necessitated the use of DNA sequences from additional genomic regions in order to infer a phylogeny for these taxa.

DNA sequences obtained from the β -tubulin gene have been employed to predict a phylogeny for closely related species in the *Gibberella fujikuroi* (Sawada) Wollenw. species complex (O'Donnell et al 1998). Several unlinked loci were used in a study by O'Donnell et al (1998), and the β -tubulin gene yielded the most variation of all areas sequenced, possibly making it useful for determining phylogeny in recently diverged groups. The β -tubulin gene product is an important component of microtubules, the major constituents of the cytoskeleton and mitotic spindles. The fact that mutations in this gene can confer resistance to the fungicide benomyl, implies that a significant body of sequencing data are already available for comparisons in fungi (Koenraad & Jones 1993, Yan & Dickman 1996). The utility of the β -tubulin gene sequence in determining phylogenetic relationships has also been demonstrated at various taxonomic levels (Schardl et al 1994, Tsai et al 1994, Donaldson et al 1995, Baldauf & Doolittle 1997).

Gene phylogeny may not necessarily be an accurate reflection of species phylogeny (Doyle 1992, Maddison 1997). One problem could be posed by the presence of several copies of the β -tubulin gene under different selection constraints. Several copies of this gene have been encountered in plants (Snustad et al 1992), and more than one copy have also been reported in fungi, such as the two divergent copies in *Colletotrichum graminicola* (Panaccione & Hanau 1990), and five in *Epichloë* species (Schardl et al 1994, Tsai et al 1994). However, ascomycetes generally appear to have lower copy numbers and several species have been described with only one β -tubulin gene (Neff et al 1983, Orbach et al 1986, Smith et al 1988).

The present study of *Calonectria* has shown that the gene phylogeny obtained from the 5' end of the β -tubulin is concordant with that obtained from the ITS flanking sequences of the 5.8 S rRNA gene, as well as the HMG box of the *MAT*-2 gene (Part 3). These results indicated that the β -tubulin gene could be suitable for determining the phylogeny of this closely related group of fungi. The aims of this study were to utilise the DNA sequences of the 5' end of the β -tubulin gene in order to obtain a phylogeny for species in *Calonectria*, and to investigate species relationships at a larger scale than in previous studies. Inclusion of biological species such as those forming part of the *Cy. candelabrum* species complex (Part 2) would also enable a

comparison of the morphological, phylogenetic and biological species concepts in this genus.

Materials and Methods

Isolates

Strains were either obtained from culture collections (Table I) or isolated from infected plant material or soil samples (Crous et al 1997b). These have been deposited in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U).

Isolation of DNA

Single conidial isolates were grown on malt extract agar (MEA) (Biolab, Midrand, South Africa) plates. Mycelial mats were cut from the plates using a sterile scalpel and ground to a powder with liquid nitrogen and a mortar and pestle. Approximately 40 mg of ground mycelia was added to 2 ml microtubes containing 600 µl of extraction buffer. The extraction buffer consisted of 1% SDS, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 100 mM EDTA. The subsequent protocol was followed as suggested for the Wizard Genomic DNA Purification kit (Promega, Madison, U.S.A.).

PCR amplifications and sequencing

A wide variety of isolates were used for sequencing (Table I). Reactions (total volume 25 µl) comprised of 1.5 units Biotaq (Bioline, London, U.K.) with the buffer as recommended by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM MgCl₂, 0.5 µM primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA as target. These were performed on a Rapidcycler (Idaho Technology Idaho, U.S.A.). Reaction conditions consisted of the following: an initial denaturation for 2 min at 96°C, followed by 30 cycles of 15 s at 96°C, 30 s at 55°C and 35 s at 75°C with a slope of 1.0. A last elongation step of 2 min at 75°C was included. A 600 bp fragment encompassing the first three introns and exons and part of the fourth exon of the β -tubulin gene was amplified with the use of primers T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). PCR fragments were sequenced as described previously (Part 3). DNA sequences of isolates of *Cy. floridanum*, *Cy. spathiphylli* and a number of unknowns (CBS 413.67, STE-U 599, 682, 1150, 1484, 2712, 2350, IMI and IMI 354529, UFV 76) previously sequenced by J.C. Kang was also included in this study for a more complete analysis.

Table 1. Isolates of *Cylindrocladium* spp. studied.

anamorph	Teleomorph	No.	Collector	Substrate	Origin	Date isolated
<i>C. vesiculatum</i>	<i>Ca. vesiculata</i>	ATCC 38226	S.A. Alfieri	<i>Ilex vomitoria</i>	Florida, U.S.A.	1971
<i>C. candelabrum</i>	<i>Ca. scoparia</i>	STE-U 1674	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil	Jul. 1990
		STE-U 1677	A.C. Alfenas	<i>Eucalyptus</i> sp.	Amazonas, Brazil	1991
		STE-U 1951	A.C. Alfenas	<i>Eucalyptus</i> sp.	Brazil	Jun. 1998
		UFV 89	A.C. Alfenas	<i>Eucalyptus</i> sp.	Brazil	1990
<i>C. citri</i>	Unknown	CBS 186.36	H.S. Fawcett	<i>Citrus sinensis</i>	Florida, U.S.A.	Jan. 1932
<i>C. colhounii</i>	<i>Ca. colhounii</i>	STE-U 681	M.J. Wingfield	Soil	Thailand	Nov. 1993
		STE-U 705	M.J. Wingfield	Soil	KwaZulu-Natal, S. Africa	Nov. 1993
		STE-U 1237	P.W. Crous	<i>Eucalyptus</i> sp.	KwaZulu-Natal, S. Africa	Oct. 1995
		STE-U 1339	M.J. Wingfield	Soil	Indonesia	Mar. 1996
<i>C. curvisporum</i>	Unknown	STE-U 763	P.W. Crous	Soil	Madagascar	Apr. 1994
		STE-U 765	P.W. Crous	Soil	Madagascar	Apr. 1994
<i>C. flexuosum</i>	<i>Ca. clavata</i>	STE-U 2536	N.E. El-Gholl	<i>Callistemon viminalis</i>	Florida, U.S.A.	Apr. 1978
<i>C. floridanum</i>	<i>Ca. kyotensis</i>	ATCC 18834	T. Terashita	<i>Robinia pseudoacacia</i>	Japan	1968
		ATCC 18882	R.H. Morrison	Peach roots	Florida, U.S.A.	1967
		CBS 413.67	W. Gerlach	<i>Paphiopedilum callosum</i>	Celle, Germany	Oct. 1967
		STE-U 682	M.J. Wingfield	Soil	Thailand	Aug. 1993
		STE-U 2350	M.J. Wingfield	Soil	Hong Kong	1998
		IMI 354528	M. Aragaki	<i>Araucaria heterophylla</i>	Hawaii	1987
		IMI 354529	M. Aragaki	<i>Araucaria heterophylla</i>	Hawaii	1987
		UFV 76	A.C. Alfenas	<i>Pinus</i> sp.	Canada	1990
<i>C. gracile</i>	Unknown	ATCC 22833	C.S. Hodges	<i>Pinus caribaea</i>	Brazil	Mar. 1971
		IMI 167580	A. Peerally	<i>Camellia sinensis</i>	Mauritius	1970
		PC 551197	Bugnicourt	<i>Argyrea splendens</i>	Vietnam	1937
		STE-U 623	M.J. Wingfield	Soil	Amazonas, Brazil	Apr. 1993
		STE-U 1586	P.W. Crous	Soil	Amazonas, Brazil	1996
<i>C. graciloideum</i>	<i>Ca. gracilipes</i>	STE-U 1153	M.J. Wingfield	Soil	Colombia	Jun. 1996
<i>C. hawsworthii</i>	Unknown	MUCL 30866	A. Peerally	<i>Nelumbo necifera</i>	Mauritius	1990
<i>C. macroconidiale</i>	<i>Ca. macroconidialis</i>	STE-U 307	P.W. Crous	<i>Eucalyptus grandis</i>	Mpumalanga, S. Africa	Mar. 1990
		STE-U 413	P.W. Crous	Soil	Mpumalanga, S. Africa	May 1990
<i>C. heptaseptatum</i>	Unknown	FTCC 1002	N.E. El-Gholl	<i>Rumohrae adiantiformis</i>	Florida, U.S.A.	Unknown
		FTCC 1003	N.E. El-Gholl	<i>Rumohrae adiantiformis</i>	Florida, U.S.A.	Unknown
		STE-U 2344	N.E. El-Gholl	<i>Rumohrae adiantiformis</i>	Florida, U.S.A.	Mar. 1999
<i>C. insulare</i>	<i>Ca. insularis</i>	STE-U 616	M.J. Wingfield	Soil	Amazonas, Brazil	Apr. 1993
		STE-U 768	P.W. Crous	Soil	Madagascar	Apr. 1994
		STE-U 954	M.J. Wingfield	Soil	Veracruz, Mexico	Apr. 1994
<i>C. leucothoes</i>	Unknown	ATCC 64824	N.E. El-Gholl	<i>Leucothoe axillaris</i>	Florida, U.S.A.	1988
		P97.2605	N.E. El-Gholl	<i>Leucothoe</i> sp.	Florida, U.S.A.	1997
<i>C. mexicanum</i>	<i>Ca. mexicana</i>	STE-U 927	M.J. Wingfield	Soil	Yucatan, Mexico	Apr. 1994
		STE-U 941	M.J. Wingfield	Soil	Holpechén, Mexico	Apr. 1994
<i>C. multiseptatum</i>	<i>Ca. multiseptata</i>	STE-U 1589	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia	Jan. 1997
		STE-U 1602	M.J. Wingfield	<i>Eucalyptus</i> sp.	Indonesia	Jan. 1997
<i>C. naviculatum</i>	<i>Ca. naviculata</i>	STE-U 627	M.J. Wingfield	Soil	Amazonas, Brazil	Apr. 1993
		STE-U 628	M.J. Wingfield	Soil	Amazonas, Brazil	Apr. 1993
<i>C. ovatum</i>	<i>Ca. ovata</i>	UFV 90	M.J. Wingfield	Soil	Amazonas, Brazil	1990
<i>C. parasiticum</i>	<i>Ca. ilicicola</i>	ATCC 46133	S.A. Alfieri	<i>Cissus rhombifolia</i>	Florida, U.S.A.	1981
		CBS 190.50	K.B. Boedijn	<i>Solanum tuberosum</i>	Java, Indonesia	Feb. 1948
			J. Reitsma			
<i>C. pauciramosum</i>	<i>Ca. pauciramosa</i>	STE-U 723	M.J. Wingfield	Soil	Colombia	Jan. 1994
		STE-U 416	S. de Buisson	<i>Eucalyptus grandis</i>	N. Province	Jun. 1990
		STE-U 972	P.W. Crous	Soil	W. Cape	Nov. 1994
		STE-U 925	M.J. Wingfield	Soil	Santa Catarina, Brazil	Apr. 1994
<i>C. penicilloides</i>	Unknown	CBS 174.55	M. Ookubu	<i>Prunus</i> sp.	Hatizyo, Japan	Jan. 1952
<i>C. pseudogratile</i>	<i>Ca. gracilis</i>	AR 2677	A.Y. Rossman	<i>Manilkara</i> sp.	Amazonas, Brazil	Unknown
		STE-U 1588	P.W. Crous	Soil	Amazonas, Brazil	1997
<i>C. pteridis</i>	<i>Ca. pteridis</i>	STE-U 2190	P.W. Crous	<i>Eucalyptus</i> sp.	Amazonas, Brazil	Oct. 1996
		STE-U 2869	P.W. Crous	<i>Eucalyptus</i> sp.	Brazil	1997
		UFV 43	J.C. Dianese	Unknown	Minas Gerais, Brazil	Unknown

Table 1. Isolates of *Cylindrocladium* spp. studied (continued).

amorph	Teleomorph	No.	Collector	Substrate	Origin	Date isolated
<i>quinqueseptatum</i>	<i>Ca. quinqueseptata</i>	ATCC 16550	Unknown	<i>Scolopendrium</i> sp.	Solomon Islands	1965
		STE-U 516	M.J. Wingfield	<i>Eucalyptus</i> sp.	Thailand	Aug. 1992
		STE-U 759	P.W. Crous	<i>Eucalyptus</i> sp.	Madagascar	Jan. 1994
<i>spathiphylli</i>	<i>Ca. spathiphylli</i>	ATCC 44730	S.A. Alfieri	<i>Spathiphyllum</i> sp.	Florida, U.S.A.	1982
		STE-U 1624	M.J. Wingfield	Soil	Ecuador	Jun. 1997
		STE-U 1641	M.J. Wingfield	Soil	Ecuador	Jun. 1997
		STE-U 2186	K.I. Kavowas	<i>Heliconia psitacorum</i>	Florida, U.S.A.	1986
		STE-U 2188	A. Thompson	<i>Spathiphyllum</i> sp.	Mpumalanga, S. Africa	Feb. 1998
<i>rumohrae</i>	<i>Ca. rumohrae</i>	UFV 215	A.C. Alfenas	<i>Rumohrae adiantiformis</i>	Panama	Jan. 1997
		UFV 218	A.C. Alfenas	<i>Rumohrae adiantiformis</i>	Panama	Jan. 1997
		STE-U 1603	R. Pieters	<i>Adiantum</i> sp.	The Netherlands	Jan. 1996
<i>scoparium</i>	<i>Ca. morganii</i>	ATCC 38227	S.A. Alfieri	<i>Mahonia bealei</i>	Florida, U.S.A.	1970
		ATCC 46300	D.M. Benson	<i>Leucothoe catesbaei</i>	North Carolina, U.S.A.	1981
		STE-U 1720	N.E. El-Gholl	<i>Rosa</i> sp.	Florida, U.S.A.	Jan. 1998
		STE-U 1722	N.E. El-Gholl	<i>Dodonea viscosa</i>	Florida, U.S.A.	Jan. 1998
<i>spathulatum</i>	<i>Ca. spathulata</i>	AR 1844	C.S. Hodges	<i>Eucalyptus grandis</i>	Minas Gerais, Brazil	Unknown
		ATCC 62616	N.E. El-Gholl	<i>Eucalyptus viminalis</i>	Brazil	1985
<i>theae</i>	<i>Ca. indusiata</i>	ATCC 48895	N.E. El-Gholl	<i>Rhododendron</i> sp.	Florida, U.S.A.	Unknown
		UFV 16	N.E. El-Gholl	<i>Rhododendron</i> sp.	Minas Gerais, Brazil	Unknown
<i>variabile</i>	<i>Ca. variabilis</i>	AR 2675	F.C. de Albuquerque	<i>Didymopanax morototoni</i>	Pará, Brazil	1990
		UFV 28	A.C. Alfenas	<i>Eucalyptus</i> sp.	Minas Gerais, Brazil	Unknown
<i>Cylindrocladium</i> sp.	<i>Calonectria</i> sp.	STE-U 2321	J. Taylor	Soil	Madagascar	Dec. 1998
		STE-U 2322	J. Roux	Soil	Congo	Dec. 1998
		STE-U 2347	N.E. El-Gholl	<i>Rumohrae adiantiformis</i>	Florida, U.S.A.	May 1999
		STE-U 599	P.W. Crous	Soil	Brazil	Jan. 1993
		STE-U 1150	M.J. Wingfield	Soil	Colombia	Jan. 1995
		STE-U 1484	P.W. Crous	Soil	Brazil	Aug. 1998
		STE-U 2712	M.J. Wingfield	<i>Eucalyptus grandis</i>	Colombia	1998

Phylogenetic analysis

Alignments of sequences were done with the computer package Malign version 2.7 (Wheeler & Gladstein 1991) and assessed manually and are included in the Appendix (Alignment 5). A number of isolates Phylogenetic analysis of aligned DNA sequences was performed using PAUP* 4.0b1 (Swofford 1998) and printed with the help of Treeview Version 1.5 (Page 1996). The large number of indels found in the three non-coding regions proved to be problematic for alignment. Twenty-four highly ambiguous characters in the third intron (base pair 542-567) were excluded from the analysis. Analyses were also done both with gaps treated as a "missing" and as a "fifth base" in PAUP*4.0b1. Finally, in order to limit the influence of large gaps consisting of several characters only the first character of a multi-character gap was coded. Subsequent gap characters were coded as missing data. The former treatment yielded 104 and the latter 1 most parsimonious trees after addition of 1000 random sequences using a heuristic search algorithm. Confidence intervals were determined using 1000 bootstrap replications. Decay indices were determined with Autodecay Version 4.0 (Eriksson 1998). Data sets were also assessed by using neighbor-joining with uncorrected ("p") distance methods and ties were broken

randomly in PAUP* 4.0b1. The outgroup sequence was obtained from GenBank (*Fusarium subglutinans*, accession number, U34417).

Results

Sequences of the complete open reading frame of the β -tubulin gene from *Gibberella fujikuroi* (*tub2*) were obtained from GenBank (Accession no. U27303). After comparisons with the partial gene sequences obtained from *Calonectria* a similar arrangement for the coding and non-coding regions was observed in this species. Both of these species had three introns and exons in the genomic DNA area amplified. This confirmed the close relationship between these species.

Due to the number of sequences used and the high amount of possible most parsimonious trees, the neighbor-joining analysis method of Saitou and Nei (1987) was applied to a complete data set containing DNA sequence data sets obtained from more than one isolate per species, where possible. This data set consisted of 92 ingroup taxa with 582 total characters of which 316 were parsimony informative. The PCR fragments of the partial β -tubulin gene obtained from the different *Cylindrocladium* species had a variation of 31 base pairs in length. The regions used for analysis differed from 509 to 540 base pairs, while the outgroup *F. subglutinans* had the shortest length (494 base pairs). The tree obtained after 1000 bootstrap repetitions showed a number of clades within two larger clades (Fig. 1). Clade A included the largest number of species, as well as subclades 1-8. Clade B encompassed a smaller number of species (clades 9 and 10). In addition to this, it was evident that most isolates of the same morphological species grouped together with strong bootstrap support.

In order to perform a cladistic analysis a reduced data set of taxa containing a single isolate of each morphological species was used. This data set consisted of 30 ingroup taxa with 579 characters. Twenty four highly ambiguous characters at the end of the third intron were excluded. This left 170 variable parsimony informative characters. A heuristic search with 1000 random additions yielded 104 most parsimonious trees when gaps were treated as missing and a single most parsimonious tree when gaps were treated as a fifth base (Fig. 2). The topology of all these trees were similar, but lower bootstrap support for branches were found when gaps were ignored. The topology of the tree in Fig. 2 is mainly concordant with that of the neighbor-joining tree in Fig. 1 and shows close relationships for the same morphological species. Two large clades are again evident from this tree.

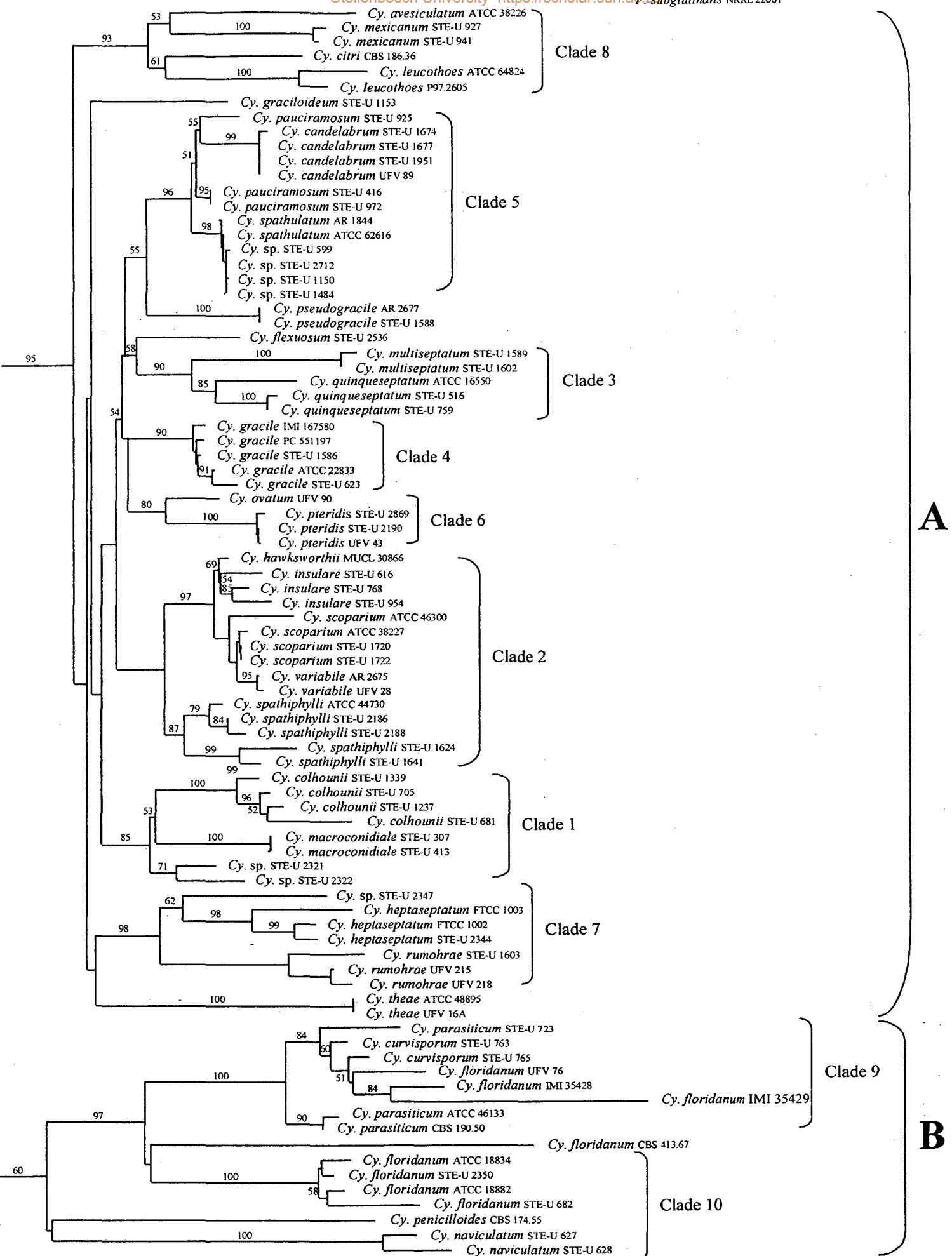


Fig.1. Neighbor-joining tree of total group of taxa. Bootstrap values were assessed after 1000 repetitions and values above 50 % are shown. Clades supported by bootstrap values are indicated by brackets. A *Fusarium subglutinans* sequence (Genbank accession number: U34417) was used as outgroup.

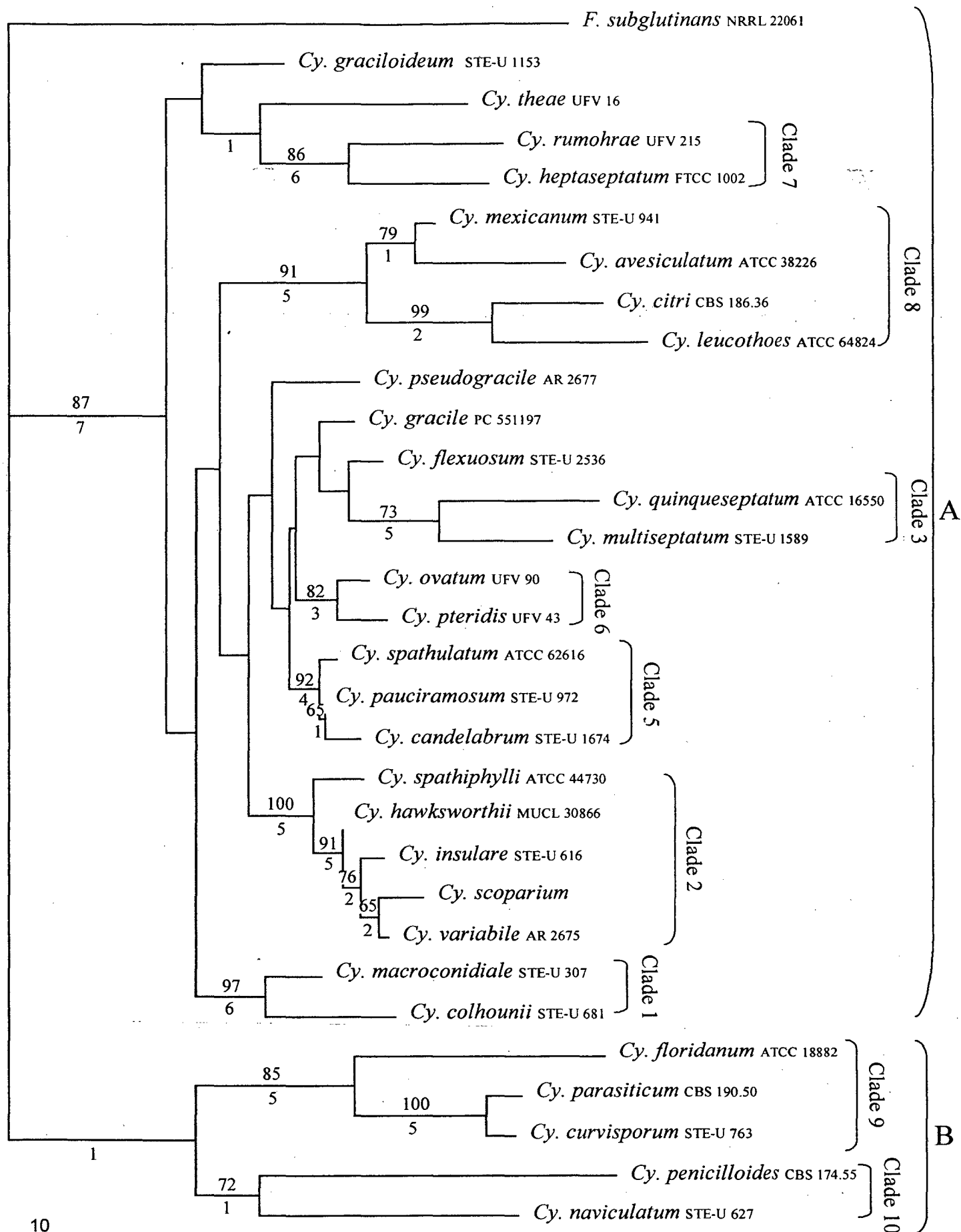


Fig. 2. Parsimonious tree obtained from a subset of *Calonectria* isolates. The most parsimonious tree (958 steps CI = 0.568, RI = 0.551, RC = 0.313) generated with a heuristic algorithm in PAUP* version 4.0b1 from aligned sequences of the 5' end of the β -tubulin gene. Ten steps are indicated by the bar. Gaps were treated as a fifth base. Clade stability was assessed with 1000 bootstrap replications and values above 50 % are shown. Decay indices are shown below branches. A *Fusarium subglutinans* sequence (Genbank accession number: U34417) was used as outgroup.

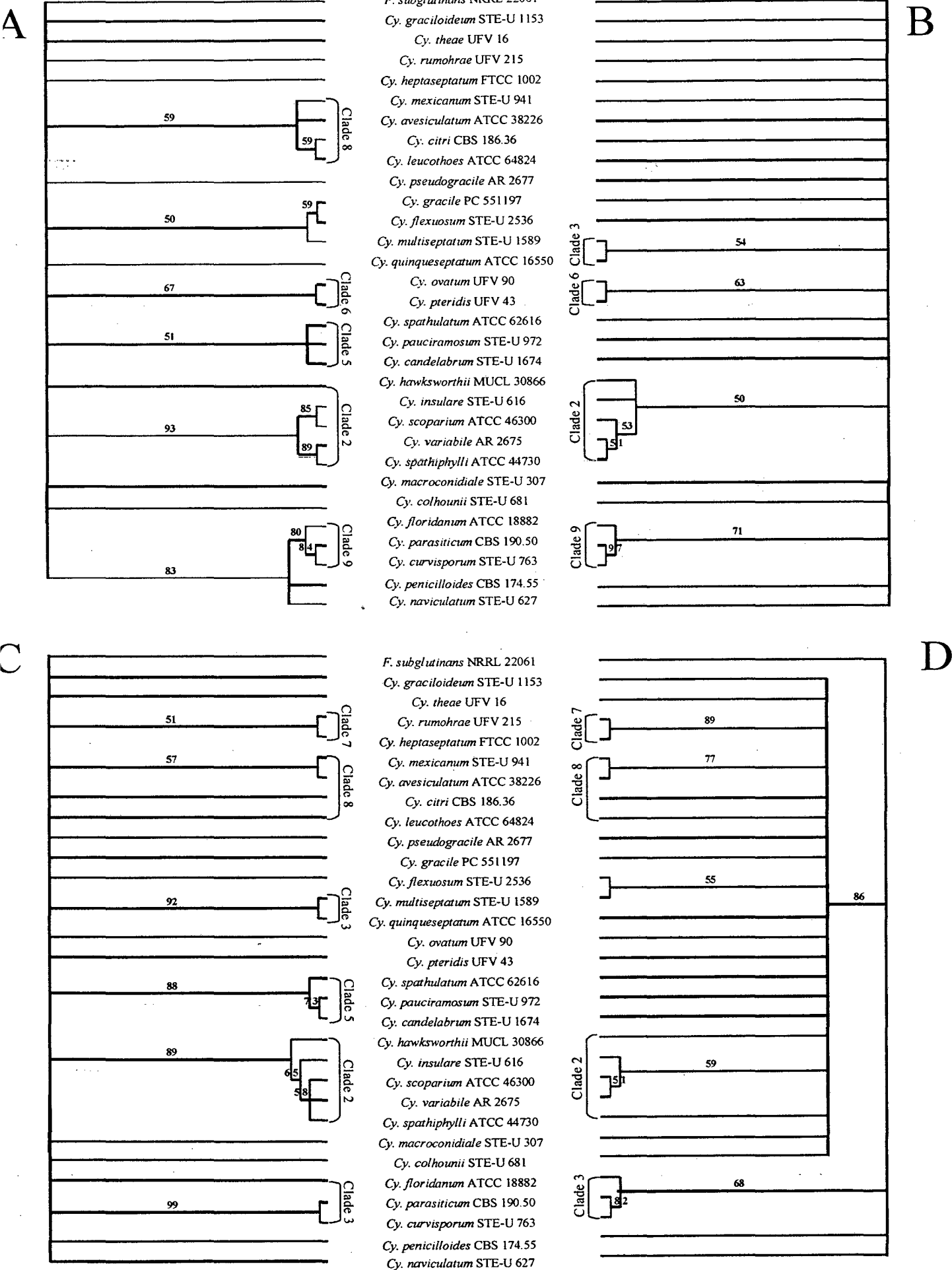


Fig. 3. Neighbor-joining trees of selected areas in the β -tubulin DNA sequence data set. A. intron 1 B. intron 2 C. intron 3 D. coding sequence. Clade stability was assessed after 1000 repetitions.

Several smaller clades are apparent that generally conform to those observed in the neighbor-joining tree (Fig. 1). However, like the neighbor-joining tree no strong bootstrap support was evident for relationships between several clades.

In order to test whether specific areas in the DNA fragments sequenced specifically influenced the compositions of the dendrograms (Figs. 1 and 2) separate analyses for each of the introns found in the area of β -tubulin sequenced were performed. The large number of possible most parsimonious topologies made a cladistic analysis difficult and the neighbor-joining method was used (Fig. 3A-D). The first intron consisted of 167 characters, with 96 informative sites, the second had 74 characters with 52 informative sites and the third 112 characters with 78 informative sites. The protein coding area was also subjected to the same analysis (Fig. 3D). This data set consisted of 230 characters with 45 informative sites.

The separate analyses yielded a number of clades weakly supported by bootstrap values (Fig. 3A-D). A higher number of clades observed in Figs. 1 and 2 were supported in analyses of the three different introns. The protein sequences also provided support for the two large clades A and B observed in Figs. 1 and 2. Only one clade (clade 1) was supported in the neighbor-joining (Fig. 1) and heuristic trees (Fig. 2) but not in any of the separate analyses (Fig. 3). Several clades were supported in more than one of the areas analysed, but in some instances a smaller number of species were supported e.g. clade 2 (Fig. 3A-D). Finally, a close relationship is suggested between previously unsupported clades such as *Cy. multiseptatum* and *Cy. flexuosum* (Fig. 3A, D). The bootstrap support for this relationship was low in Fig 3 and although the most parsimonious tree in Fig. 2 supported this relationship, it was not supported by bootstrap values in that analysis.

Discussion

This study presents the first attempt to consider the phylogeny of all described species in the genus *Cylindrocladium*. Previous studies have used smaller subsets of isolates in order to investigate morphologically defined groups, such as those species with multiseptate conidia (Crous et al 1999) and heterothallic species with small conidia (Part 3). Several of these studies have corroborated morphological species concepts (Crous et al 1997b, Crous et al 1999, Part 3) and also showed the presence of additional genetic groups within morphologically defined taxa (Part 2). Some of these seemingly closely related fungi have also been found to group

distantly in DNA sequence based phylogenies, such as *Cy. mexicanum* Crous & C.L. Schoch, originally described as part of the *Cy. candelabrum* species complex (Part 3). The current study tests whether species in the genus have evolutionary relationships not realised in previous, less encompassing studies.

The β -tubulin DNA sequence based phylogeny of *Cylindrocladium* species has both confirmed some, but contradicted other taxonomic concepts for the genus. Several clades received strong bootstrap support (Figs. 1-3). However, the relationships between most of these indicated clades were not supported by statistical data and remained unresolved. In spite of this, several conclusions could be made.

In the first large clade (A) the isolate representing *Cy. graciloideum* Crous & G.R.A. Mchau formed a distinct branch, basal to clades 1-6. Clade 1 comprises species from the *Cy. colhounii* Peerally species complex. *Cy. macroconidiale* Crous et al was previously described as a large-spored variant of *Cy. colhounii* (Crous & Wingfield 1994) and has only recently been proposed as a separate species based on sequence and morphological data (Crous et al 1999). Isolates STE-U 2231 and 2232 could not be identified as either *Cy. macroconidiale* or *Cy. colhounii* by means of sequence data and possibly represent one or two new phylogenetic species. Although these isolates had smaller conidia than other isolates of *Cy. colhounii*, their taxonomic placement remained unclear. They clustered distantly from the other isolates, but are retained as *Cy. colhounii* for the present.

Cylindrocladium species with ellipsoid and globose vesicles were grouped within clade 2 (Fig. 1). Two well-supported subclades could also be distinguished. In the first, the close relationship between isolates of *Cy. scoparium* and *Cy. variabile* Crous et al was surprising, since they both have distinctive characters. *Cy. scoparium* is heterothallic with exclusively one-septate macroconidia, while *Cy. variabile* is a homothallic species with microconidia and predominantly three-septate macroconidia. Other morphologically distinctive species included in this group are *Cy. insulare* Crous & C.L. Schoch and *Cy. hawksworthii* Peerally. The most notable difference between these two species is the presence of curved conidia in *Cy. hawksworthii*. Preliminary mating studies confirmed the DNA sequence based phylogeny and showed both species to be sexually compatible (results not shown). This calls into question the value of curved conidia as a distinguishing character for species of *Cylindrocladium*. The second sub-clade consisted of isolates of *Cy. spathiphylli* Schoult. et al This cluster contained isolates with two mating strategies

(homo- and heterothallic). Although they are morphologically indistinct, the groups containing isolates with either of these mating strategies could clearly be differentiated based on DNA sequence comparisons.

A number of clades (3, 6 and 7) contained isolates from two distinct morphological species with bootstrap support. In one of these clades (clade 7) isolate P99.0545 had intermediate morphological features between *Cy. heptaseptatum* Sobers et al and *Cy. rumohrae* El-Gholl & Alfenas. The statistical analysis (Fig. 1) only showed low bootstrap support for a similarity with *Cy. heptaseptatum*. This species could thus not be identified with any certainty and could not be delimited as a new species. The species clustering in clades 3 and 7 shared morphological characters such as multiseptate conidia and clavate vesicles. However, the isolates of *Cy. ovatum* El-Gholl et al and *Cy. pteridis* F.A. Wolf in clade 6 had clear differences in vesicle shape and spore size and were never previously considered to be closely related.

Clade 4 contained isolates previously identified as *Cy. gracile* (Bugnic.) Boesew. and *Cy. clavatum* Hodges & L.C. May (ATCC 22833). Variation between various strains were evident. However, these species were recently synonymised on morphological characters (Crous et al 1999) and these results supported this.

Clade 5 contained a number of species with spathulate to obpyriform vesicles. Isolates of two distinct biological species, *Cy. candelabrum* and *Cy. pauciramosum* Crous & C.L. Schoch were included in this group. *Cy. pauciramosum* isolates also exhibited prominent intraspecies variation and one isolate (STE-U 925) showed similarities to *Cy. candelabrum*. In addition to this, a large number of unknown isolates that were obtained from various locations in South America, tentatively identified as a possible new species, clustered strongly with isolates of *Cy. spathulatum* El-Gholl et al. These isolates were provisionally identified as *Cy. reteaudii* (STE-U 1150 and STE-U 2712) and were found to be associated with a serious disease of eucalypts in Colombia. After sequence comparisons were made they were found to share the same sequences with those obtained from the type species of *Cy. spathulatum*. On the basis of statistical analysis and re-examination of their morphological characters they were reclassified as *Cy. spathulatum*.

A number of species with variable morphological features were represented in clade 8. Some of these species had umbonate vesicles, (*Cy. mexicanum* and *Cy. leucothoes* El-Gholl et al), but the additional two species had distinctly clavate [*Cy.*

citri (Fawcett & Klotz) Boedijn & Reitsma] or clavate to avesiculate vesicles (*Cy. avesiculatum* Gill et al). As was true for clade 6, these species were not previously considered to be closely related based on morphology.

In addition to those species forming part of well supported clades, several species could not be positioned on the tree with any statistical support (Figs. 1 and 2). Isolates representing the morphological species *Cy. flexuosum* Crous, *Cy. theae* (Petch) Subram. and *Cy. pseudogracile* Crous grouped separately within the first large clade, but without any strong indications of their relationships to other species. However, their distinctiveness as separate species were supported by these data.

The second large clade (B) consisted of clades 9 and 10, as well as additional groups consisting of isolates of a single species. Clade 9 contained isolates from three morphological species - *Cy. floridanum*, *Cy. parasiticum* Crous et al and *Cy. curvisporum* Crous & Victor. The differentiation for those isolates seen in Fig. 1 is not distinct and will have to be re-evaluated in future. All of these species have sphaeropendunculate vesicles, with differences in conidial shape and septation. The second clade (clade 10) consisted of the type culture of *Cy. floridanum* (ATCC 18882) and additional isolates identified as *Cy. floridanum*. Another isolate of *Cy. floridanum* (UFV 76) also clustered separately from any of the clades. These data distinguished at least three distinctive groups within *Cy. floridanum*, supporting the results of previous studies in this complex (Jeng et al 1997, Victor et al 1997).

This study represents the first instance where it has been possible to investigate the phylogenetic relationship of *Cy. penicilloides* (Tubaki) Tubaki to other species. *Cy. penicilloides* was initially described without any mention of its vesicle morphology (Tubaki 1958). Furthermore its ex-type culture is infertile and no dried specimens could be located. Data from the present study confirmed that it is a distinct species without clear indication of its phylogenetic placement. Similarly, isolates of *Cy. naviculatum* Crous & M.J. Wingf. formed part of the larger clade (B) but could not be placed phylogenetically.

This study has provided an opportunity to compare the morphological species and biological concepts previously used for species in *Cylindrocladium* with a phylogenetic species concept. A similar species concept, based on propositions made earlier by Nixon and Wheeler (1990) has previously been applied on isolates in the *Gibberella fujikuroi* complex by O'Donnell et al (1998). The biological species

described in Part 2 provided a convenient “bench mark” enabling comparison with other species concepts. The existence of biological species within the confines of morphological species delimitations of *Cylindrocladium* was discussed earlier and only slight morphological differentiations was possible for these species (Part 2). Results of Part 3 confirmed the delimitations of these biological concepts as was also validated in the current study. The only morphological characters that agreed to some extent with the DNA based phylogeny presented here was vesicle shape. This was not surprising, as Crous and Wingfield (1994) showed that vesicle shape is an important character, but it had to be assessed under controlled conditions. However, most clades did not exclusively have one vesicle shape and the clavate shape appeared to be present in several clades with unresolved relationships.

In general, this study has emphasised that most morphological and biological species of *Cylindrocladium* represent separate phylogenetic entities. These data were also helpful in confirming identifications of isolates with intermediate or indeterminate morphological characters. Several questions, however, remain unresolved. This includes the close phylogenetic relationships seen between some species previously considered to be distinct based on morphological characters.

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6. Phylogeny of *Calonectria* and selected hypocrealean genera with cylindrical macroconidia*

Abstract

Calonectria is characterised by having brightly coloured, warty perithecia and *Cylindrocladium* anamorphs. Other hypocrealean genera in this complex have a similar perithecial anatomy and anamorph morphology, except those of *Cylindrocladiella* spp. which are smooth-walled and clearly distinct. The aim of this study was to employ DNA sequence analysis to determine the phylogeny of *Calonectria* to other hypocrealean genera with cylindrical macroconidia. The taxonomy of species in *Cylindrocladiella* was also investigated. *Calonectria* was found to form a monophyletic lineage, and this was also true for the anamorph genera *Cylindrocladiella*, *Cylindrocarpon*, *Curvocladium*, *Gliocephalotrichum*, *Gliocladiopsis* and *Xenocylindrocladium*. Although some of these genera have been associated with nectriaceous teleomorphs, *Nectria* sensu stricto is restricted to species with *Tubercularia* anamorphs. Based on molecular data and the distinct anamorph form genera, new teleomorph genera are proposed for *Cylindrocladiella* (*Nectricladiella*), *Gliocladiopsis* (*Glionectria*) and *Xenocylindrocladium* (*Xenocalonectria*). The data also provide support for recognition of previously erected holomorphs for *Cylindrocarpon* (*Neonectria*) and *Gliocephalotrichum* (*Leuconectria*). To date no teleomorph has been reported for *Curvocladium*, although our results suggest that *C. cigneum* is closely related to *Xenocalonectria*. Eight species of *Cylindrocladiella* are recognised, with two having teleomorphs in *Nectricladiella*, namely *N. camelliae* (*Ce. microcylindrica*) and *N. infestans* (*Ce. infestans*).

Introduction

The ascomycete order *Hypocreales* includes fungi found in a variety of ecological niches that are of agricultural, medical and industrial importance. These fungi are characterized by unitunicate asci produced in typically ostiolate, brightly or lightly coloured perithecia, hyaline ascospores and a hamathecium of apical paraphyses

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that disintegrate at maturity (Rogerson 1970, Rossman et al 1999). It is noteworthy that a large number of anamorph genera are associated with the *Hypocreales* (Samuels & Seifert 1987). These can be described as moniliaceous and typically phialidic; conidia are held in lightly to brightly coloured slime (Samuels & Seifert 1987). The importance of anamorph morphology in taxonomic studies is emphasized by the fact that in many economically important species this form is more frequently encountered than the teleomorph, and is thus often the only way to identify a species.

In the *Hypocreales*, *Nectria* (Fr.) Fr. has included more species than any other genus, with more than 600 described. Traditionally all species having fleshy, uniloculate ascocarps with a hypocrealean centrum with hyaline, non-apiculate, bicellular ascospores, and phialidic anamorphs have been included in *Nectria* (Rossman 1993). In this generic definition, ascospore morphology and septation dominated. Other genera were segregated from *Nectria* on the basis of single characters, including ascospore septation and pigmentation, and synnematus anamorphs. *Calonectria* De Not. (Ca.), one of the segregate genera that is of special interest to the current work, was described for species having multiseptate ascospores (Saccardo 1883).

Booth (1959) was the first to use a combination of characters that included anatomy of the perithecial wall, ecology and anamorphs in describing informal taxonomic groups in *Nectria sensu lato*. Subsequent authors (e.g. Samuels 1976, Samuels et al 1991, Brayford & Samuels 1993, Samuels & Brayford 1993) followed Booth in recognizing informal groups within the large genus *Nectria*. In a recent revision of genera of the *Hypocreales* (Rossman et al 1999), many of these groups were given generic status and additional genera were described. *Nectria sensu stricto* was restricted to the type species, *Nectria cinnabarina* (Tode : Fr.) Fr., and species similar to it. Rossman et al (1999) split the large and polyphyletic genus *Nectria* into several smaller genera within two families, the Nectriaceae and the Bionectriaceae. *Calonectria* was included in the Nectriaceae, but was differentiated from *Nectria sensu stricto* on the basis of ascocarp morphology and anatomy, the occurrence of a *Cylindrocladium* Morgan (Cy.) anamorph, and basic differences in biology. Although the singular character of ascospore morphology was regarded as less important (Rossman 1983, Crous & Wingfield 1994), ascospores of *Calonectria* are distinct from those of *Nectria*.

In a study based on the sequence alignments of the nuclear large-subunit ribosomal DNA obtained from several genera in the *Hypocreales*, Rehner and Samuels (1995) found some species to group together with *Calonectria*. These authors showed that species of *Calonectria* grouped closely to *Leuconectria clusiae* Rossman et al (anamorph: *Gliocephalotrichum bulbilium* J.J. Ellis & Hesselt.), as well as to *Nectria radiculicola* Gerlach & L. Nilsson [anamorph: *Cylindrocarpon destructans* (Zinssm.) Scholten], with two typical species of *Nectria*, *N. pseudotrichia* Berk. & M.A. Curtis [anamorph: *Tubercularia lateritia* (Berk.) Seifert] and *N. cinnabarina*, forming part of this subclade, but grouping more distantly. This phylogeny generally confirmed morphological observations, where similarities were found between the *Gliocephalotrichum* and *Cylindrocladium* anamorphs of *Leuconectria* and *Calonectria* (Rossman & Samuels 1993), the most notable similarities being the formation of cylindrical conidia and brown pigment diffusing in the agar.

In addition to *Gliocephalotrichum*, several other anamorph form-genera are similar to *Cylindrocladium* in producing cylindrical macroconidia, phialidic conidiogenous cells and slimy conidia. Among these are *Cylindrocladiella* Boesew. (Ce.), *Gliocladiopsis* S.B. Saksena, *Xenocylindrocladium* Decock et al and *Curvocladium* Decock & Crous. Of these, only *Cylindrocladiella* (Boesewinkel 1982) and *Xenocylindrocladium* (Decock et al 1997) have been linked to teleomorphs, both forming part of *Nectria sensu lato*. We have included representatives of these genera in the present evaluation of holomorphs having cylindrical conidia.

Anamorphs have assumed an increasingly important role in the delimitation of genera of the *Hypocreales* (Rossman et al 1999), to the extent that they have replaced ascospores as the single most important phylogenetically informative character. The advent of data derived from sequences of the rDNA gene has provided independent support for the phylogenetic significance of anamorphs. These data have indicated that some anamorphs that have the 'hypocrealean phenotype' do, in fact, cluster with sexually reproducing genera of the *Hypocreales* (e.g. Spatafora & Blackwell 1993, Rehner & Samuels 1994, 1995, Glenn et al 1996; O'Donnell et al 1998). Moreover, individual anamorph species that are either not known to reproduce sexually, or that are encountered frequently in the absence of sexual reproduction (i.e. perithecia) can be phylogenetically related to sexually reproducing holomorphs (Kuhls et al 1996, 1997).

Additional anamorph genera and species are likely to be linked to the *Hypocreales* as additional DNA sequence data become available. Considering this and recent trends in favour of discarding the phenetically based form-genera of the deuteromycetes (Sutton, 1993), Rossman (1993, this volume) proposed that each hypocrealean teleomorph genus should potentially be linked to one anamorph genus. This is in step with a more holomorphic approach, encompassing both teleomorph and anamorph (Hawksworth, 1993). However, the generic concepts as currently applied still have a strong influence from Saccardo's original taxonomic system (Rossman, 1996, this volume), and detailed cultural and molecular studies are required to clarify anamorph/teleomorph relationships and attain a genus for genus phylogeny as far as possible.

The revision of genera of the *Hypocreales* proposed by Rossman et al (1999) was acknowledged by the authors as a 'starting point' rather than a final statement on the *Hypocreales*. They acknowledged that many of the genera that they delimited are still poly- or paraphyletic, and that new genera remain to be described as new species are discovered through exploration. Most of the genera that were recognized by Rossman et al (1999) have not been assessed using DNA characters. In the present work we consider holomorphs of nectriaceous ascomycetes that have cylindrical conidia whose anamorphs are classified in several genera. These ascomycetes are united by the formation of small, red perithecia that are situated on a small basal stroma, occur singly or in clusters, and have pigments that change colour in 3% KOH. Species of *Calonectria* are characterized by warted perithecia, and clavate, long-stemmed asci without a visible apical discharge mechanism, and large ($\leq 25 \mu\text{m}$), 1- to multiseptate, hyaline, smooth, fusiform ascospores with obtuse ends that aggregate in the upper third of the ascus. Based on teleomorph morphology alone, however, species of *Calonectria* can only be identified to species complexes, and the anamorph is required for identification at species level. The perithecial wall anatomy of *Calonectria* is not unique, but is also shared by teleomorphs of some *Cylindrocarpon* (*destructans*-complex), *Xenocylindrocladium* and *Gliocladiopsis* species. The latter are primarily distinguished from *Calonectria* based on their ascus and ascospore morphology. That said, teleomorphs of the latter three genera would be difficult if not impossible to distinguish without knowledge of their respective anamorphs. In contrast, the teleomorphs of *Cylindrocladiella* spp. are quite distinct from those discussed above, as they have a smooth, relatively thin-walled *Cosmospora*-like perithecia that easily collapse laterally when dry, a less well-developed basal stroma, and smaller ascospores.

Materials and Methods

Isolates

Strains were either obtained from other culture collections or isolated from infected plant material or soil samples (Crous *et al.*, 1997) and deposited in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (acronym STE-U, Table 1). Hypocrealean genera are abbreviated as follows: *Calonectria* – Ca.; *Cylindrocladium* – Cy.; *Cylindrocladiella* – Ce., and *Cylindrocarpon* – Co.

Acronyms used to denote culture collections of institutions and individuals from which isolates were obtained include: ATCC – American Type Culture Collection, Virginia, U.S.A.; A.R. – A.R. (A. Y. Rossman), C. T. R. (C. T. Rogerson) and G. J. S. (G. J. Samuels), United States Department of Agriculture, A.R.S., Beltsville, Maryland, U.S.A.; IMI – CABI Bioscience, Bakeham Lane, Egham, U.K.; IMUR – Institute of Mycology, University of Recife, Brazil; MUCL – Mycothèque, Laboratoire de Mycologie Systématique et Appliquée, Université Louvain-la-Neuve, Belgium; STE-U – (see above), and UFV – (A. C. Alfenas), Department of Plant Pathology, University of Viçosa, Viçosa, Minas Gerais, Brazil.

Morphological comparisons

Isolates were cultured on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa), plated onto carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous *et al.*, 1992), incubated at 25°C under near-ultraviolet light, and examined after 7 d. Only material growing on carnation leaves was examined. Mounts were prepared in lactophenol, examined using Nomarski interference phase contrast and bright-field phase contrast microscopy, and measurements made at x 1000 magnification. The 95% confidence intervals were determined from at least 30 observations and the minimum and maximum ranges given in parentheses. Cardinal temperature requirements for growth and cultural characteristics were determined after 6 d on MEA, using procedures described by Crous and Wingfield (1994). Colony colours were coded according to Rayner (1970). Sections of perithecia were cut at 10 µm thickness on a CM1100 Cryostat microtome (Leica, Heidelberg, Germany).

Table 1. Isolates used in this study.

Anamorph	Teleomorph	Original no.	Collector	Host	Origin
<i>Cylindrocladium scoparium</i>	<i>Calonectria morganii</i>	ATCC 38227	S.A. Alfieri	<i>Mahonia bealei</i>	Florida, U.S.A.
		ATCC 46300	D.M. Benson	<i>Leucothoe catesbaei</i>	North Carolina, U.S.A.
<i>Cylindrocladium floridanum</i>	<i>Calonectria kyotensis</i>	ATCC 18882	R.H. Morrison	Peach roots	Florida, U.S.A.
		ATCC 18834	T. Terashita	<i>Robinia pseudoacacia</i>	Japan
<i>Cylindrocladium candelabrum</i>	<i>Calonectria scoparia</i>	STE-U 1677	A.C. Alfenas	<i>Eucalyptus</i> sp.	Amazonas, Brazil
		STE-U 1674	A.C. Alfenas	<i>Eucalyptus</i> sp.	Bahia, Brazil
<i>Cylindrocladium multiseptatum</i>	<i>Calonectria multiseptata</i>	STE-U 1589	M.J. Wingfield	<i>Eucalyptus</i> sp.	Sumatra, Indonesia
		STE-U 1602	M.J. Wingfield	<i>Eucalyptus</i> sp.	Sumatra, Indonesia
<i>Cylindrocladiella novae-zelandiae</i>	None described	ATCC 44815	H.J. Boesewinkel	<i>Rhododendron indicum</i>	New Zealand
<i>Cylindrocladiella elegans</i>	None described	STE-U 518	P.W. Crous	Litter	Western Cape, South Africa
<i>Cylindrocladiella parva</i>	None described	ATCC 28272	H.J. Boesewinkel	<i>Telopea speciosissima</i>	New Zealand
		STE-U 373	P.W. Crous	<i>Pinus radiata</i>	Western Cape, South Africa
<i>Cylindrocladiella peruviana</i>	None described	IMUR 1843	M.P. Herrera	Ants	Brazil
		STE-U 395	P.W. Crous	<i>Acacia mearnsii</i>	KwaZulu Natal, South Africa
<i>Cylindrocladiella lageniformis</i>	None described	UFV 115	A.C. Alfenas	<i>Eucalyptus</i> sp.	Brazil
<i>Cylindrocladiella infestans</i>	<i>Nectricladiella infestans</i>	ATCC 44816	H.J. Boesewinkel	<i>Pinus pinea</i>	New Zealand
		IMI 299376	K.B. Boedijn & J. Reitsma	<i>Arenga pinnata</i>	Indonesia
		STE-U 708	M.J. Wingfield	Soil	Hong Kong
		STE-U 2319	J.E. Taylor	Soil	Madagascar
<i>Cylindrocladiella microcylindrica</i>	<i>Nectricladiella camelliae</i>	ATCC 38571	W.A. Shipton	<i>Pinus pinea</i>	Australia
		STE-U 683	M.J. Wingfield	Soil	Thailand
		STE-U 918	Unknown	Soil	Salta, Argentina
<i>Cylindrocladiella camelliae</i>	None described	STE-U 234	P.W. Crous	<i>Eucalyptus grandis</i>	Northern Province, South Africa
		STE-U 277	P.W. Crous	<i>Eucalyptus grandis</i>	Northern Province, South Africa
<i>Cylindrocarpon macroconidialis</i>	<i>Neonectria radicola</i> var. <i>macroconidialis</i>	GJS 83-162	G.J. Samuels	<i>Astelia</i> sp.	New Zealand
<i>Cylindrocarpon destructans</i>	<i>Neonectria radicola</i> var. <i>radicola</i>	AR 2553	A.Y. Rossman	Bark	Venezuela
		CTR 71-322	G.J. Samuels	Host unknown	Venezuela
<i>Cylindrocarpon destructans</i> var. <i>coprosmae</i>	<i>Neonectria radicola</i> var. <i>coprosmae</i>	CTR 73-152	G.J. Samuels	<i>Cosmospora</i> sp.	New Zealand
		GJS_85-182	G.J. Samuels	Unknown	New Zealand
<i>Gliocladiopsis tenuis</i>	<i>Glionectria tenuis</i>	STE-U 706	M.J. Wingfield	Soil	Hong Kong
<i>Gliocladiopsis sumatrensis</i>	None described	STE-U 1351	M.J. Wingfield	Soil	Sumatra, Indonesia
<i>Gliocladiopsis irregularis</i>	None described	STE-U 718	A.C. Alfenas	Soil	Sumatra, Indonesia
<i>Curvocladium cigneum</i>	None described	STE-U 1595	C. Decock	Leaf of angiosperm	French Guiana
<i>Xenocylindrocladium serpens</i>	<i>Xenocalonectria serpens</i>	STE-U 1144	G.L. Hennebert	Bark of unknown tree	Ecuador

DNA extraction and sequencing

Single conidial isolates were grown on MEA plates. Mycelial mats were removed from the plates and ground to a powder with the help of liquid nitrogen and a mortar and pestle. Approximately 40 mg of ground mycelium was added to 2 ml microtubes containing 600 µl of extraction buffer. The extraction buffer consisted of 1% SDS, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 100 mM EDTA. The subsequent protocol was followed as suggested for the Wizard Genomic DNA Purification kit (Promega, Madison, U.S.A.).

Reactions (total volume 25 µl) comprised of 1.5 units Biotaq (Bioline, London, U.K.) with the buffer as recommended by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM MgCl₂, 0.5 µM primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA as target. Reactions were performed on a Rapidcycler (Idaho Technology, Idaho, U.S.A.). Reaction conditions consisted of the following: an initial denaturation for 2 min at 96°C, followed by 30 cycles of 15 s at 96°C, 30 s at 55°C and 35 s at 75°C with a slope of 1.0. A last elongation step of 2 min at 75°C was included. DNA was amplified using the primers ITS1 and ITS4 (White et al 1990). The region amplified was the 5.8S ribosomal gene and the two internal transcribed spacers (ITS1 and ITS2). An approximately 540 bp fragment was amplified. The PCR products were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). Sequencing conditions were as described in Part 2.

Phylogenetic analysis

Sequences were aligned with the computer package Malign version 2.7 (Wheeler & Gladstein 1991) and adjusted manually. Phylogenetic analysis of aligned DNA sequences was performed using PAUP* Version 4.0b1 (Swofford 1998) and printed with the help of Treeview Version 1.5 (Page 1996). In order to limit the influence of large gaps consisting of several characters only the first character of a multi-character gap was coded. Subsequent gap characters were coded as missing data. Having done this, the analyses were done treating these single character gaps as fifth characters. A number of strains representing different species in each genus were selected for the generic analysis (Fig. 1). In this instance a heuristic search option with 1000 random addition sequences was used. The analysis for species with *Cylindrocladiella* anamorphs were performed using the branch and bound search option. Confidence intervals were determined using 1000 bootstrap

replications in all cases. Decay indices were determined with Autodecay Version 4.0 (Eriksson 1998). A partition homogeneity test was performed in PAUP* Version 4.0b1 in order to test whether phylogenies obtained from the ITS and β -tubulin data sets differed significantly. This was done heuristically with 1000 replications. Data sets were also analyzed by using Neighbor-Joining with uncorrected ("p") and maximum-likelihood distance methods in PAUP* Version 4.0b1.

Taxonomy

A phylogenetic analysis of all species in this study, based on the DNA sequence of the two flanking internally transcribed spacers (ITS1 and ITS2) and the 5.8S ribosomal RNA gene is shown in Fig. 1. When gaps were coded as missing the number of possible most parsimonious trees was in excess of 1000. With gaps treated as a fifth character only one most parsimonious tree was found. No difference in the number of most parsimonious trees was found when all subsequent gap characters after the first gap character was coded as missing. However, this reduced the number of parsimony informative sites from 163 to 139. All species clustered in accordance with their distinctive anamorphs and the groupings evident from this are discussed in more detail below.

Calonectria/Cylindrocladium, Curvocladium, Nectria/Xenocylindrocladium

The type species of *Calonectria* is *Ca. daldiniana* De Not., now considered a synonym of *Ca. pyrochroa* (Desm.) Sacc. (Rossman 1979a). *Calonectria* encompasses species with brightly coloured ascocarps that become red in 3% KOH solution (KOH+), have a thick perithecial wall that consists of large cells and have a darkened stromatic base. Ascospores of *Calonectria* tend to be longer than 25 μ m, are fusiform, and usually phragmosporous. *Cylindrocladium* spp. have been linked to *Calonectria* teleomorphs exclusively (Rossman 1993). Rossman (1979b) redispersed many species ascribed to *Calonectria*.

The anamorph genus *Cylindrocladium* was originally based on *C. scoparium* Morgan, a species that was collected from a dead pod of honey locust (*Gleditsia triacanthos* L.) in Ohio, U.S.A. (Morgan 1892). Species in this genus are well-known plant pathogens and have been isolated from all continents in tropical and subtropical zones world-wide (Crous & Wingfield 1994). Species concepts in *Cylindrocladium* have been defined based on the dimensions and septation of conidia, phialide shape, stipe length, cultural characteristics, as well as the shape and diameter of the

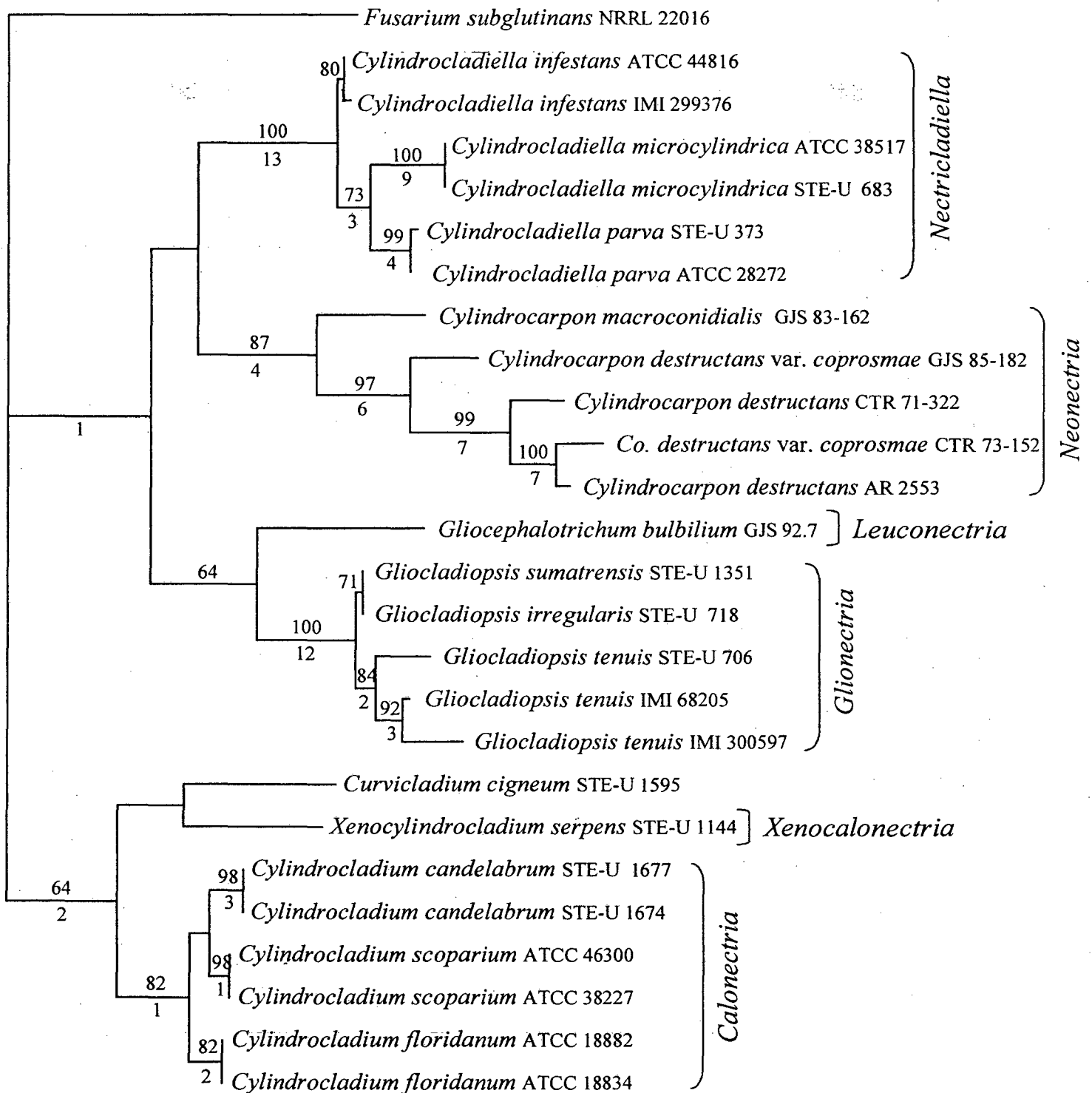


Fig. 1. One of four most parsimonious trees (405 steps CI = 0.681 RC = 0.554 RI = 0.812) obtained with a heuristic search in PAUP* version 4.0b1 and 1000 random addition sequences. Bootstrap values are shown above branches and decay indices below. Characters used were based on a data set comprising of ITS1 and 2 as well as the 5.8S ribosomal gene DNA sequences.

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Taxonomy

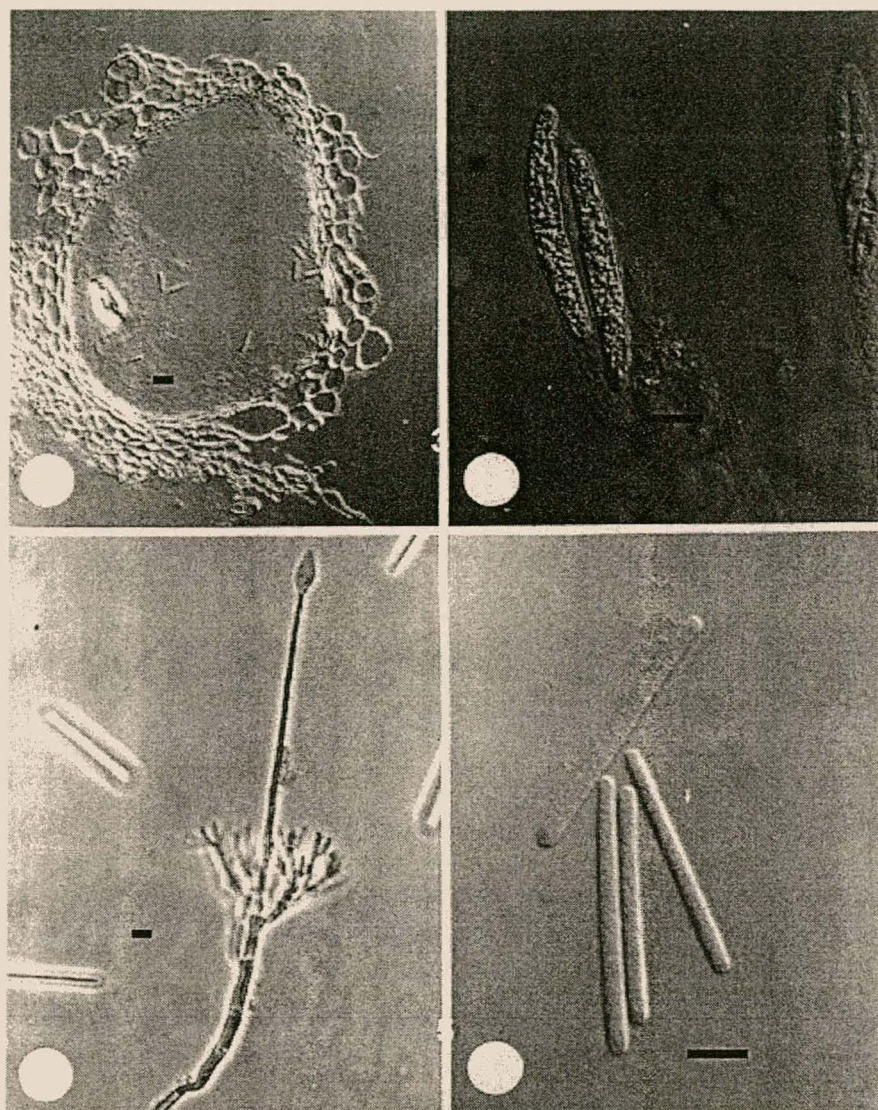
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terminal vesicle found on stipes emanating from the conidiophores (Figs. 2-5) (Crous & Wingfield, 1994).



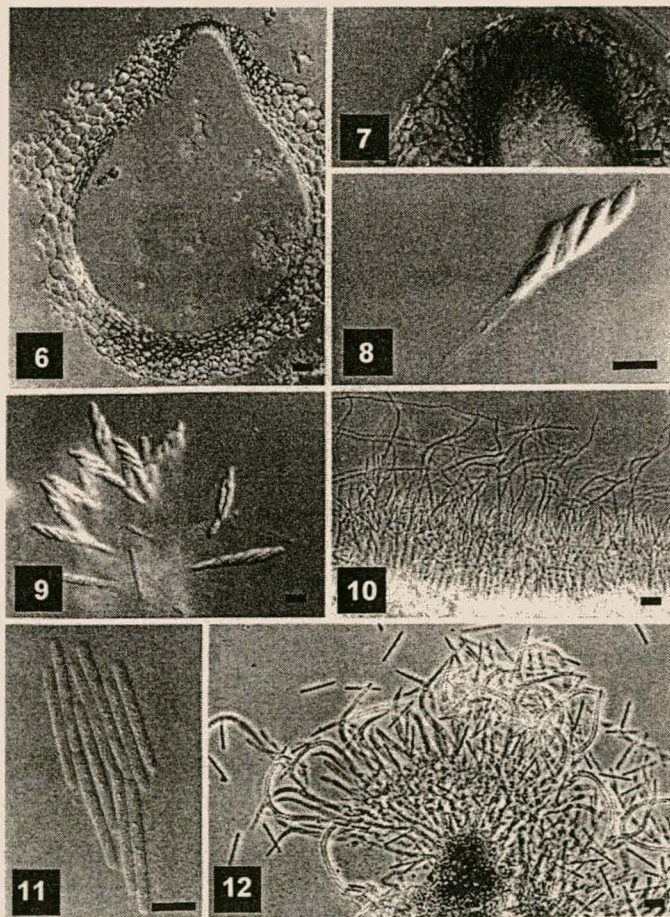
Figs. 2-5. *Calonectria mexicana* and its anamorph *Cylindrocladium mexicanum*. 2. Vertical section through a perithecium. 3. Asci with ascospores. 4. Conidiophore with extending stipe and terminal vesicle. 5. One-septate conidia. Bars = 10 μ m.

Previous studies, using sequence data obtained from ITS, β -tubulin and the HMG box of *MAT-2*, showed that concordant phylogenies could be derived from the gene trees based on different loci in this genus (Crous et al 1999, Part 3). Some of these species were also shown to have interfertility barriers, thus complying with a biological species concept (Part 1). Although these results generally coincided with morphological species concepts, in some cases several phylogenetic species (based on DNA sequence data) and biological species could be described within the parameters of a morphological species.

Two new hyphomycete genera with penicillate conidiophores and unique stipe elongations were described that also appeared to be morphologically closely related

to *Cylindrocladium* (Decock et al 1997, Decock & Crous 1998). *Xenocylindrocladium serpens* was described from Ecuador as the type species of this genus, while its teleomorph, distinct from *Calonectria*, was described in *Nectria* as *N. serpens* Decock et al. (Decock et al 1997). A similar fungus, *Curvocladium cigneum* Decock & Crous, was later described as yet another new genus in this complex, characterized by curved, rough, sparsely septate stipe extensions (Decock & Crous 1998). No teleomorph has yet been reported for *Curvocladium* (Fig. 12).

The species of *Calonectria* included in this study all produced *Cylindrocladium* anamorphs characteristic of this genus, and formed a clearly distinct clade, strongly supported by high bootstrap values (Fig. 1). The *Calonectria* clade was shown to be closely related to *Xenocylindrocladium* and *Curvocladium* (Fig. 1). Their close proximity to *Calonectria* suggests a shared ancestor. This hypothesis will still have to be tested further, however, using additional gene trees.



Figs. 6-12. *Nectria serpens* and *Curvocladium cigneum*. 6-11. *Xenocalonectria serpens* and its anamorph *Xenocylindrocladium serpens*. 6. Vertical section through perithecial wall. 7. Ostiolar region of perithecial wall. 8-9. Cylindrical asci with apical apparatus. 10. Conidiophores with stipe extensions. 11. One-septate conidia. *eum.* 12. Conidiophores and conidia of *Curvocladium cigneum*. Bars = 10 μ m.

Based on the phylogenetic distance shown in Fig. 1, as well as distinct morphological differences in the anamorph of *Xenocylindrocladium*, we propose the following new holomorph genus:

Xenocalonectria Crous & C.L. Schoch gen. nov.

Anamorphe: *Xenocylindrocladium* Decock, Hennebert & Crous

Typus: *Xenocalonectria serpens* (Decock, Hennebert & Crous) Crous & C.L. Schoch
Perithecia superficialia, solitaria vel aggregata, globosa ad subglobosa, verrucosa, lutea usque ad rubra, cum basi obscure rubra stromatica, KOH+; pariete perithecii ex duabus regionibus composito: strato exteriori ex *textura globulosa* cum parietibus crassitunicata, strato interiore ex cellulis compressis *texturae angularis*; periphyses ostioli hyalinae, tubulares cum apicibus rotundatis. Asci unitunicati, octospori, cylindrici basi elongata, apice applanato et apparatu apicali refringente. Ascosporae in parte superiore asci aggregatae, hyalinae, late vel anguste ellipsoideae, leves, medio uniseptatae.

Perithecia superficial, solitary or in clusters, globose to subglobose, warted, yellow to red and with a dark red stromatic base, KOH+; perithecial wall consisting of two regions: outer layer of thick-walled *textura globulosa*, inner layer of compressed cells of *textura angularis*; ostiolar periphyses hyaline, tubular with rounded ends. Asci unitunicate, 8-spored, cylindrical, with long basal stalks, a flattened apex, and a refractive apical apparatus. Ascospores aggregated in the upper third of the ascus, hyaline, broadly to narrowly ellipsoidal, smooth, medianly 1-septate. Anamorph is *Xenocylindrocladium*.

Xenocalonectria serpens (Decock, Hennebert & Crous) Crous & C.L. Schoch, *comb.nov.* — (Figs. 6-11).

≡ *Nectria serpens* Decock, Hennebert & Crous, Mycol. Res. 101: 788. 1997.

Anamorph: *Xenocylindrocladium serpens* Decock, Hennebert & Crous, Mycol. Res. 101: 788. 1997.

Holotypes. ECUADOR. SUCUMBIOS: Reserva de Producción Faunística, Cuyabeno, Tierra firme, bark of a fallen tree trunk, Jul. 1993, G.L. Hennebert, MUCL 39315a, holotype of teleomorph, MUCL 39315b, holotype of anamorph (culture ex-type: MUCL 39315 = STE-U 1144).

This species was described in full by Decock *et al.* (1997). Ascospores aggregated in the upper third of the ascus, hyaline, broadly to narrowly ellipsoidal, smooth, with granular contents, (8-)12-20(-25) x 4-5(-6) µm, medianly 1-septate, becoming constricted at the septum, and developing up to 2 septa with age. Macroconidia

cylindrical, hyaline, straight with rounded ends, 1-septate, (24-)27-33(-36) x 2.5-3(-3.5) μm .

Cultures. Colony colour (reverse) 13K, amber brown (Rayner 1970). Chlamydospores in extensive numbers, with medium to extensive sporulation on aerial mycelium.

Cardinal temperature requirements for growth. Minimum above 5°C, optimum 25-30°C, maximum below 35°C.

Substrate. Bark of fallen trees.

Nectria/Cylindrocarpon

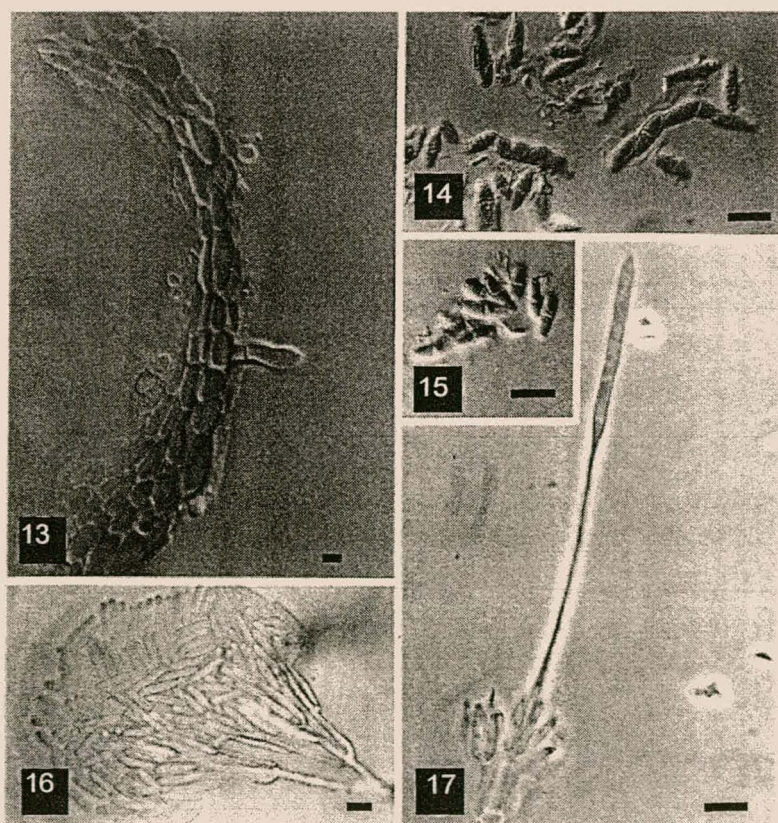
Perithecial anatomy in *N. radicola* and its relatives is similar to that of *Calonectria* (Samuels & Brayford 1990). Samuels and Seifert (1987) commented on the similarity between *Cylindrocladium* and the *Cylindrocarpon* Wollenw. anamorphs of *N. radicola* and closely related species. *Calonectria* and the nectriaceous species centred around *N. radicola* are distinguished primarily by the respective occurrence of *Cylindrocladium* and *Cylindrocarpon* anamorphs, as well as on their distinct ascus and ascospore morphology (Samuels & Brayford 1990). Ascospores of the *radicola*-group are, however, much smaller than those of *Calonectria* spp. Rossman et al (1999) referred many holomorphs having *Cylindrocarpon* anamorphs to *Neonectria* Wr. *Nectria radicola* (which was not transferred to *Neonectria*) and its relatives, all of which have *Cylindrocarpon* anamorphs, cluster in a clade (Fig. 1) that is sister to *Cylindrocladiella*. Whether *N. radicola* is representative of all holomorphs having *Cylindrocarpon* anamorphs (*Neonectria*) is currently being evaluated (F. Mantiri & G. Samuels pers. comm.).

Nectria/Cylindrocladiella

A new anamorph genus was erected in 1982 to accommodate five small-spored species of *Cylindrocladium* (Boesewinkel, 1982). This new genus, *Cylindrocladiella*, was reported to have different conidiophore branching patterns, conidial shapes, dimensions as well as cultural characteristics. The recognition of *Nectria camelliae* Shipton as the teleomorph for one of these species made a strong case for the delimitation of the new genus. More recent studies have confirmed the genera *Cylindrocladium* and *Cylindrocladiella* to be distinct (Crous & Wingfield, 1993; Crous

et al 1994, Victor et al 1998). Samuels et al (1991) allocated *N. camelliae* (anamorph: *Ce. infestans*) to *Nectria* subg. *Dialonectria*, while Rossman et al (1999), in a re-evaluation of the group, placed it in *Cosmospora* as *C. camelliae* (Shipton) Rossman & Samuels, based on its perithecial morphology and anatomy. As presently defined by Rossman et al (1999), *Cosmospora* is heterogeneous in having diverse anamorphs, including *Cylindrocladiella*. In comparison to *Calonectria* spp., the perithecial wall of *Cosmospora camelliae* is smooth, narrow, and its ascospores are much smaller.

Victor et al (1998) recognised seven species in *Cylindrocladiella*. All these species could be distinguished based on RFLP and AT-DNA data, as well as morphology. The AT-DNA data showed differences in the profiles of the ex-type isolates of *Cosmospora camelliae* (ATCC 38571; teleomorph) and *Ce. infestans* (ATCC 44816; anamorph). One restriction enzyme also showed differences in the RFLP profiles, but cultural and morphological characters have shown little variation other than conidial length (Victor et al 1998).



Figs. 13-17. *Nectriadiella infestans* and its *Cylindrocladiella microcylindrica* anamorph. 13. Vertical setum through a perithecial wall, showing smooth wall and hyphal mite, brown seta. 14. Broken asci and ascospores. 15. Ascospores. 16. Conidium and conidia. 17. Conidiophore with stipe extensions and terminal cylindrical vesicle. Bars = 10 μ m.

The *Nectria/Cylindrocladiella* clade has strong bootstrap support (Fig. 1). Relationships between the groupings *Nectria/Cylindrocladiella* and *Neonectria* are equivocal because the clade that includes these two groups received only weak

bootstrap support. However, both groups are strongly supported as separate entities in accordance with their different anamorphs. Two areas of the genome were utilised in order to investigate relationships in those species with *Cylindrocladiella* anamorphs. When the phylogenies derived from data sets obtained from the ITS regions flanking the 5.8S ribosomal RNA gene as well as the 5' end of the β -tubulin gene were compared in a partition homogeneity test, they were not found to differ significantly ($P = 0.33$, where $P < 0.05$ denotes significance) (Fig. 18). The number of parsimony informative sites in the ITS data set (25) were much less than those in the β -tubulin data set (109). A similar trend occurred in *Calonectria* species (Part 2).

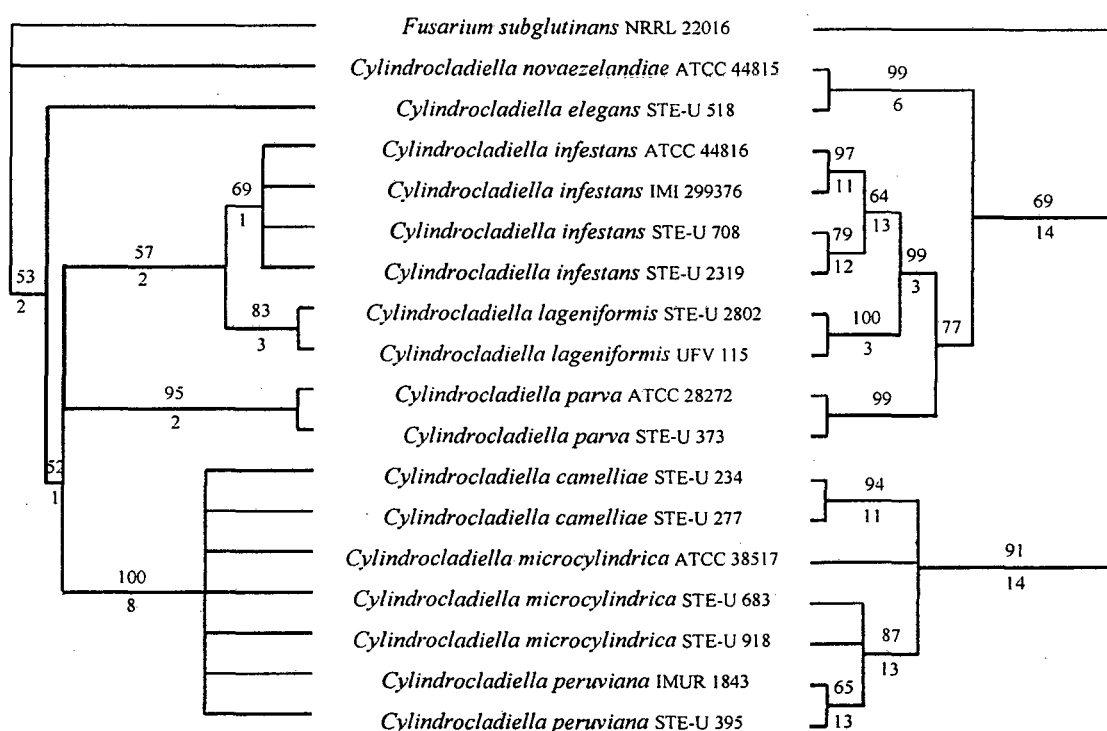


Fig. 18. Concordance of two most parsimonious trees obtained from the ITS (left) and β -tubulin (right) data sets. The ITS data set yielded one most parsimonious tree and β -tubulin yielded four. Trees were obtained with a branch and bound search in PAUP* version 4.0b1 and 1000 random addition sequences. Bootstrap values are shown above branches and decay indices below.

The DNA sequence data of both ITS and β -tubulin loci have shown clear differences between two groups of isolates identified as *Ce. infestans* (Fig. 18). One group is characterised by the culture on which the name *Cosmospora camelliae* is based, while the other is characterised by the culture on which the name *Ce. infestans* is based. Furthermore, an isolate from the “anamorph type grouping”, recently obtained from Madagascar, produced a teleomorph in culture. The clear differences shown in the molecular data, based on two DNA sequence data sets and the

previous characters described by Victor *et al.* (1998), suggest that *Ce. infestans* contains more than one genetically distinct taxon. These are described as new below.

Cylindrocladiella microcylindrica, *Ce. peruviana* (Bat., J.L. Bezerra & S. Herrera) Boesew. and *Ce. camelliae* (Venkataram & C.S.V. Ram) Boesew. were shown to cluster together in the tree based on the ITS data (Fig. 18). Likewise, the β -tubulin data set showed support for a distinct grouping of these species, but could not differentiate between them (Fig. 18). Previously, Crous and Wingfield (1993) synonymized *Ce. peruviana* with *Ce. camelliae* based on similarity in morphology. Conidiophores of both of these species have ellipsoid to lanceolate vesicles and similar conidial dimensions as well as similar temperature growth relationships, but Victor *et al.* (1998) separated them based on differences in RFLP profiles as well as vesicle width and taper. The data in Figs. 1 and 18 show that this close relationship is also reflected in the molecular characters used here. The β -tubulin data set supported their separation. Further variation in this clade based on the β -tubulin sequences was also evident. Although molecular data confirmed *Ce. camelliae* and *Ce. peruviana* to be different, isolates of the third species, *Ce. microcylindrica*, exhibited similarities to these two taxa, and more isolates will have to be studied to clearly resolve the boundaries among these species.

The relationships of the other species in the genus, *Ce. elegans* Crous & M.J. Wingf., *Ce. novaezealandiae* (Boesew.) Boesew., *Ce. lageniformis* Crous *et al.* and *Ce. parva* (P.J. Anderson) Boesew. were represented as separate entities in both data sets (Fig. 18). A close relationship between *Ce. novaezealandiae* and *Ce. elegans* is only supported by the β -tubulin data set. Based on the distinct clade of *Cylindrocladiella* species identified here, as well as their unique morphological traits, backed up by molecular data, a new holomorphic genus is proposed below.

Nectricladiella Crous & C.L. Schoch gen. nov.

Anamorphe: *Cylindrocladiella* Boesewinkel

Typus: *Nectricladiella camelliae* (Shipton) Crous & C.L. Schoch

Perithecia superficialia, solitaria, stromate basali egentes, globosa ad obpyriformia, collabentia ubi arida, levia, numerosis setis parvis ex pagina parietis perithecii orientibus; apice et corpore perithecii rubro, basi brunnea, KOH+, ostiolum ex cellulis columnaribus compositum, cum periphysibus hyalinis inconspicuis indutum; pariete

peritheciis ex 3-4 stratis texturae angularis composito cum cellulis compressis, hyalinis. Asci unitunicati, octospori, cylindrici, sessiles, tenuitunicati et apice applanato. Ascospores uniseriatae, superpositae, hyalinae, ellipsoideae ad fusiformes, cum apicibus obtusis, uniseptatae.

Perithecia superficial, solitary, basal stroma absent, globose to obpyriform, collapsing laterally when dry, smooth, with several minute, brown setae arising from the perithecial wall surface, red, KOH+; ostiole consisting of clavate cells, lined with inconspicuous, periphyses; perithecial wall consisting of a single region of 3-4 cell layers of *textura angularis*, which become hyaline and slightly flattened towards the centre. Asci unitunicate, 8-spored, cylindrical, sessile, thin-walled, with a flattened apex, and a refractive apical apparatus. Ascospores uniseriate, overlapping, hyaline, ellipsoid to fusoid with obtuse ends, smooth, 1-septate. Anamorph is *Cylindrocladiella*.

Nectricladiella camelliae (Shipton) Crous & C.L. Schoch comb. nov.

≡ *Calonectria camelliae* Shipton & C. Booth, Trans. Br. Mycol. Soc. 69: 59. 1977 (nom. nud.).

≡ *Calonectria camelliae* Shipton, Trans. Br. Mycol. Soc. 72: 163. 1979.

≡ *Nectria camelliae* (Shipton) Boesewinkel, Can. J. Bot. 60: 2293. 1982.

≡ *Cosmospora camelliae* (Shipton) Rossman & Samuels, Stud. Mycol. 42: 118. 1999.

Anamorph: *Cylindrocladiella microcylindrica* Crous & D. Victor sp. nov.

Etymology. *Micro* + *cylindrica*, named after its smaller conidia and cylindrical vesicles.

Holotypes. AUSTRALIA. QUEENSLAND: Fruit of a rainforest tree, W.A. Shipton, 1973, IMI 174836, holotype of teleomorph PREM 51724, holotype of anamorph (culture ex type: ATCC 38571 = STE-U 2375).

Characteribus culturae, morphologia et temperaturae provento *C. infestanti* similis sed distincta propter conidia minoria. Conidia hyalina, 1-septata, cylindracea, apicibus obtusis, (10-)12-14(-15) x 2(-3) µm.

Perithecia described in full by Shipton (1979). Ascospores hyaline, median septate, unstricted, oval to ellipsoid, 6.5-10.5 x 2.5-4 µm. Anamorph morphology and

cultural characteristics similar to those of *Ce. infestans*, but conidia shorter (10-)12-14(-15) x 2(-3) μm , than those of the former (10-)14-16(-20) x 2(-3) μm .

Cultures. Colony colour (reverse) 19D, buff yellow (Rayner 1970). Chlamydospores in medium numbers, arranged in chains.

Cardinal temperature requirements for growth. Minimum above 5°C, optimum 25°C, maximum below 35°C.

Substrate. Soil.

Distribution. Australia, Argentina, Brazil, Thailand.

Nectricladiella infestans Crous & C.L. Schoch sp. nov.

Anamorph. *Cylindrocladiella infestans* Boesewinkel, Can. J. Bot. 60: 2290. 1982.

Holotypes. MADAGASCAR: Rana, isolated from soil, J.E. Taylor, 1998, PREM 56380, holotype of teleomorph (culture ex type: STE-U 2319). NEW ZEALAND: Isolated from *Pinus pinea*, H.J. Boesewinkel, CBS 487.76, holotype of anamorph (culture ex type: ATCC 44816 = STE-U 2380).

Description. Perithecia superficialia, solitaria, sine stromate basale, globosa ad obpyriformia, 150-200 μm alta et lata, collabentia ubi arida, levia, cum numerosis setis parvis ex pagina parietis perithecii orientibus; apice et corpore perithecii rubro, base brunnea, 3% KOH + [bene agens in 3% KOH], parte superiore rubro brunnea facta, base brunneo rubra facta; ostiolum ex cellulis columnaribus compositum, cum periphysis hyalinis inconspicuis; pariete perithecii 10-15 μm lato, ex 3-4 stratis texturae angularis composito; interiore regione hymenii ex 3-4 stratis composita, cum cellulis compressis, hyalinis. Asci unitunicati, octospori, cylindrici, leviter clavati in maturitate, sessiles, cum parietibus tenuibus et apice applanato, apparatu apicale refracto, 35-60 x 4-6 μm . Ascosporae: 8 in uno asco, uniseriatae, superpositae, hyalinae, ellipsoideae ad fusiformes, cum apicibus obtusis, leves, altissimae ad medium septum vel ad regionem leviter superiorem, non constrictae, 8-10(-12) x 3-3.5 μm ; ex perithecio extantes, profusae et hyalinae. Morphologia anamorpha et characteristic in cultura *Ce. microcylindrica* similis sed cum conidiis longioribus, (10-)14-16(-20) x 2(-3) μm .

Perithecia superficial, solitary, basal stroma absent, globose to obpyriform, 150-200 µm high and thick, collapsing when dry, smooth, with several minute, brown setae arising from the perithecial wall surface; apex and perithecial body red, base brown, reacting positive in 3% KOH, upper part turning red-brown, base becoming brown-red; ostiole consisting of columnar cells, lined with inconspicuous, hyaline periphyses; perithecial wall 10-15 µm thick, consisting of 3-4 layers of *textura angularis*; inner hymenium region of 3-4 layers of flattened, hyaline cells. *Asci* unitunicate, 8-spored, cylindrical, becoming slightly clavate at maturity, sessile, thin-walled, with a flattened apex, and a refractive apical apparatus, 35-60 x 4-6 µm. *Ascospores* 8 per ascus, uniseriate, overlapping, hyaline, ellipsoid to fusoid with obtuse ends, smooth, widest at median septum or slightly above, unstricted, 8-10(-12) x 3-3.5 µm; extruding from perithecia in yellow mass. Anamorph morphology and cultural characteristics similar to those of *Ce. microcylindrica*, but conidia longer, (10-)14-16(-20) x 2(-3) µm.

Habitat. *Arenga pinnata*, *Pinus pinea*, soil.

Distribution. New Zealand, Madagascar, Hong Kong, Indonesia.

Leuconectria/Gliocephalotrichum

The similarities in perithecial anatomy between *Leuconectria* and *Calonectria* have been noted before (Rossman & Samuels 1993). Their *Gliocephalotrichum* and *Cylindrocladium* anamorphs also share several characteristics. Besides having penicillate conidiophores, cylindrical conidia, and forming chlamydospores in culture, both anamorph genera have stipe extensions, even though they originate from different areas on the conidiophores. Cultural characteristics are also similar. Furthermore, both teleomorphs have KOH+, solitary, red perithecia. Perithecia of *Leuconectria* are distinct, however, in having a white covering that is absent in species of *Calonectria*. Thus far, isolates of *Leuconectria* have been obtained from decaying leaves, fruits, or from soil, and nothing is known about their potential status as plant pathogens. It is similar to the other taxa dealt with in this paper in that they occupy similar habitats, all basically being soil fungi that converge in forming more or less similar, small, red perithecia. This is in contrast to *Cylindrocarpon sensu stricto*

(exclusive of the *radicola* complex), which are primarily lignicolous and canker-forming.

The DNA sequence data employed here support the separation of *Leuconectria* from other genera in this study (Fig. 1). The data were ambiguous about the relationship of *Leuconectria* to other genera that have cylindrical conidia while at the same time confirming the close relationship with *Calonectria* (see also Rehner & Samuels 1995).

Gliocladiopsis

The anamorph genus *Gliocladiopsis* S.B. Saksena (Saksena 1954, Crous & Peerally 1996) closely resembles *Cylindrocladium*. The type species of the genus, *G. sagariensis* S.B. Saksena was shown to be synonymous with *Cylindrocarpon tenue* Bugn. (Barron 1968). Although it had been suggested previously that the genus *Gliocladiopsis* should be retained for species lacking stipe extensions (Crous & Wingfield 1993), Watanabe (1994) synonymised it with *Cylindrocladium* based on the uncertainty of stipe formation. However, studies on *Cylindrocladium* and *Cylindrocladiella* have shown that both these genera regularly produce stipe extensions on their conidiophores under controlled conditions (Crous & Wingfield 1993, Crous & Wingfield 1994), suggesting that the non-stipe forming genus *Gliocladiopsis*, with its multi-branched, penicillate conidiophores should be retained. *Gliocladiopsis* was also represented by a clade. However, as for *Leuconectria*, the relationship of this genus to the other genera selected for this study is still uncertain, due to low bootstrap support for the phylogeny (Fig. 1). The three species described for *Gliocladiopsis* have no known teleomorphs (Saksena 1959, Crous & Wingfield 1993, Crous & Peerally 1996). The present study describes the first teleomorph associated with this genus, which was produced by homothallic cultures obtained from single conidia of *G. tenuis* (Bugn.) Crous & M.J. Wingf. (STE-U 706) on CLA after 2 mo of incubation at 22°C with a 12 h fluorescent white light / dark regime.

Herewith we propose a new holomorph genus for *Gliocladiopsis*. The new genus is based on the distances observed between other genera in the ITS DNA sequence based tree, as well as the distinct anamorph, *Gliocladiopsis*.

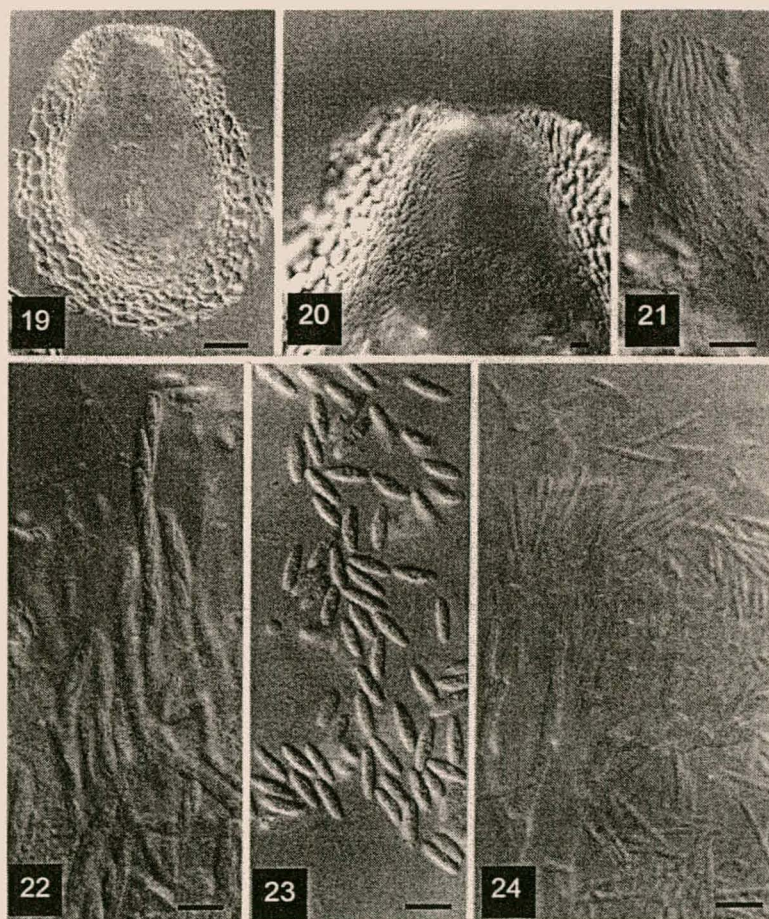
Glionectria Crous & C.L. Schoch gen. nov.

Anamorphe: *Gliocladiopsis* S.B. Saksena

Typus: *Glionectria tenuis* Crous & C.L. Schoch

Perithecia superficialia, dense gregaria, stromate tenui basali insidentia, obovoidea ad late obpyriformia, collabentia ubi arida, verrucosa, rubrobrunne basi stromatica atro-rubra, KOH+, pariete perithecii ex duabus regionibus composito: exteriore strato ex *textura globulosa* crassitunicata, interiore strato ex cellulis compressis *texturae angularis*; periphyses ostioli cylindricae, apicibus rotundatis. Asci unitunicati, octospori, cylindrici, sessiles, cum apice applanato et apparatu apicali refringente. Ascospores uniseriatae, superpositae, hyalinae, ellipsoideae, leves, medio uniseptatae.

Perithecia superficial, densely gregarious, seated on a thin basal stroma, obovoid to broadly obpyriform, collapsing laterally when dry, warted, red-brown with a dark red stromatic base, changing colour in KOH; perithecial wall consisting of two regions: outer region of thick-walled *textura globulosa*, inner region of compressed cells of *textura angularis*; ostiolar periphyses tubular with rounded ends. Asci unitunicate, 8-spored, cylindrical, sessile, with a flattened apex, and a refractive apical apparatus. Ascospores uniseriate, overlapping, hyaline, ellipsoidal, smooth, medianly 1-septate. Anamorph is *Gliocladiopsis*.



Figs. 19-24. *Glionectria tenuis* and its anamorph *Gliocladiopsis tenuis*. 19. Vertical section through a perithecium. 20,21. Ostiolar region and paraphyses. 22. Cylindrical asci with apical mechanism. 23. One-septate ascospores. 24. Conidiophore with cylindrical, 1-septate conidia. Bars = 10 μ m.

Glionectria tenuis Crous & C.L. Schoch sp. nov.

Anamorph. *Gliocladiopsis tenuis* (Bugn.) Crous & M.J. Wingf., Mycol. Res. 97: 446. 1993.

≡ *Cylindrocarpon tenue* Bugn., Encycl. Mycol. 11: 178. 1939.

≡ *Cylindrocladium tenue* (Bugn.) T. Watan., Mycologia 86: 155. 1994.

= *Gliocladiopsis sagariensis* Saksena, Mycologia 46: 663. 1954.

Holotypes. HONG KONG: Soil, M.J. Wingfield, 1993, PREM 56381, holotype of teleomorph, (culture ex type: STE-U 706). INDOCHINA (country unknown): *Indigofera* sp., F. Bugnicourt, Nov. 1936, PC 540, holotype of anamorph (culture ex type: IMI 68205 = STE-U 2403).

Description. Perithecia superficialia, dense gregaria, in stromate basale tenue sedentia, obovidea ad late obpyriformia, collabentia ubi arida, usque ad 400 µm alta et 350 µm lata, verrucosa, cum apice leviter applanata, aurantiaca, corpore et base rubro-brunnea, bene agentia in 3% KOH, apice aurantiaco-rubro facto, corpore perithecii purpureo-rubro et base atro-rubro brunnea. Regione ostiola usque ad 180 µm lata. Pariete perithecii ex duabus regionibus composito: strato exteriori ex 4-5 stratis texturae globulosae cum parietibus crassis composito, usque ad 60 µm lata, compresso ad centrum, [interiore strato] ex 3-4 stratis texturae angularis composito, usque ad 20 µm lato. Asci unitunicati, octospori, cylindrici, cum apice obtuse rotundato, sessile, cum apparatu apicale refracto, 50-80 x 4-5 µm. Ascosporae uniseriatae, superpositae, hyalinae, leves, ellipsoideae cum apicibus rotundatis, 9-12 x 2.5-3 µm, latissimae ad septum medianum, non constrictae. Conidiophora penicillata, sine extensione stipitis et sine vesiculis terminalibus. Rami conidiophori: primis ramis non septatis, 9-23 x 3-5 µm, secundis ramis non septatis, 10-18 x 2.5-3 µm, tertiis ramis non septatis, 9-14 x 2.5-3.5 µm, quartis ramis raris vel absentibus, non septatis, 9-12 x 2.5-3 µm. Phialides doliiformes ad cymbiformes ad cylindricae, 10-25 x 2.5-3 µm, in verticillis terminalibus dispositae, usque ad 7 in uno ramo, cum collulis parvis. Conidia cylindrica, hyalina, levia, cum apicibus rotundatis, medio uniseptata, (12-)16-19(-23) x 1.5-2(2.5) µm.

Perithecia superficial, densely gregarious, seated on a thin basal stroma, obovoid to broadly obpyriform, collapsing when dry, up to 400 µm high and 350 µm thick, warted, apex slightly flattened, orange, body and base red-brown, reacting positive in 3% KOH, apex becoming orange-red, perithecial body purple-red and base dark red-

brown. Ostiolar region up to 180 μm thick. Perithecial wall consisting of two regions: outer region of 4-5 layers of thick-walled *textura globulosa* up to 60 μm thick, becoming compressed towards the centrum, consisting of 3-4 layers of *textura angularis* up to 20 μm thick. *Asci* unitunicate, 8-spored, cylindrical, with a bluntly rounded apex, sessile, with a refractive apical apparatus, 50-80 x 4-5 μm . *Ascospores* uniseriate, overlapping, hyaline, smooth, ellipsoidal with rounded ends, 9-12 x 2.5-3 μm , widest at median septum, not constricted. *Conidiophores* penicillate, without stipe extensions and terminal vesicles. *Conidiophore branches*: primary branches non-septate, 9-23 x 3-5 μm , secondary branches non-septate, 10-18 x 2.5-4 μm , tertiary branches non-septate, 9-14 x 2.5-3.5 μm , quaternary branches rare to absent, non-septate, 9-12 x 2.5-3 μm . *Phialides* doliiform to cymbiform to cylindrical, 10-25 x 2.5-3 μm , arranged in terminal whorls of up to 7 per branch, with minute collarettes. *Conidia* cylindrical, hyaline, smooth, with rounded ends, medianly 1-septate, (12-)16-19(-23) x 1.5-2(-2.5) μm .

Cultures. Colony colour (reverse) 15"l, sayal brown (Rayner 1970). Chlamydospores in extensive numbers, in clearly delimited, mostly unbranched chains.

Cardinal temperature requirements for growth. Minimum above 5°C, optimum 25-30°C, maximum above 35°C.

Substrate. *Indigofera* sp., *Psidium guajava*, *Shorea robusta*, *Camellia sinensis*, *Chamaedorea elegans*, soil.

Distribution. Brazil, Colombia, Hong Kong, India, Indonesia, Thailand, U.S.A.

Key to genera of the Nectriaceae having cylindrical conidia borne in hyaline or pale yellow masses:-----

1. *Conidiophores* penicillate, mononematous..... 4
- Conidiophores* penicillate or nearly so, sporodochial or synnematus..... 2
2. Stipe extensions present, conidia in hyaline slime; extensions with one apical and basal septum, apical cell curved, pigmented, verruculose..... *Curvicladium*
2. Stipe extensions absent, conidia in hyaline or pale yellow slime;

- perithecia solitary to gregarious, warty, wall consisting of two layers; asci cylindrical, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate.....3
3. Conidiophores frequently divergent, or unbranched with a single conidiogenous cell; macroconidia straight or fusoid, 1-multiseptate, attenuating to rounded ends with a basal abscission scar; microconidia fusoid to ellipsoid, 0-1-septate.....*Neonectria* (*Cylindrocarpon*)
3. Conidiophores always penicillate with more than 2 series of branches, rarely solitary, mostly gregarious; macroconidia cylindrical with rounded ends, 1-septate, straight or curved, abscission scar inconspicuous; microconidia absent.....*Glionectria* (*Gliocladiopsis*)
4. Stipe extensions hyaline, arising above the apical penicillus..... 5
4. Stipe extensions slightly pigmented, forming below the apical penicillus; perithecia warty, wall consisting of two layers; asci narrowly clavate, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate..... *Leuconectria* (*Gliocephalotrichum*)
5. Perithecium smooth, frequently with a few reduced hyphal setae, body collapsing at maturity; asci cylindrical, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate; stipe extensions aseptate, thick-walled; conidia shorter than 25 µm; phialide collarettes convergent*Nectricladiella* (*Cylindrocladiella*)
5. Perithecium warted, consisting of two layers; asci with long basal stalk; stipe extensions multi-septate, thin-walled; conidia longer than 25 m; phialide collarettes divergent..... 6
6. Asci cylindrical with apical apparatus; ascospores 1-septate; stipe extensions spirally twisted, hyaline, smooth, vesiculate; 1-septate.....*Xenocalonectria* (*Xenocylindrocladium*)
6. Asci clavate without an apical apparatus; ascospores 1-6-septate; stipe extensions straight, terminating in a swollen vesicle of characteristic shape.....*Calonectria* (*Cylindrocladium*)

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7. Appendix - DNA alignments used

Alignment 1. Part 3. ITS1 5.8S ITS2 DNA sequence alignment of selected *Cylindrocladium* species

						60
<i>F. subglutinans</i> NRRL 22061	CCGAGTTTAC	AAC TCCCAA	CCCC-TGTGA	ACATACCAAT	T-XGTTGCCT	CGGCGGATCA
<i>Cy. candelabrum</i> STE-U 1674	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. candelabrum</i> STE-U 1677	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. candelabrum</i> STE-U 1951	XXXXXXXXXX	XXXXXXCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. insulare</i> STE-U 616	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. insulare</i> STE-U 768	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. insulare</i> STE-U 954	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. mexicanum</i> STE-U 927	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. mexicanum</i> STE-U 941	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. multiseptatum</i> STE-U 1589	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. multiseptatum</i> STE-U 1602	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. ovatum</i> STE-U 2232	XXXXXXXXXX	XXXXXXCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. ovatum</i> UFV 90	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. pauciramosum</i> STE-U 416	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. pauciramosum</i> STE-U 925	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. pauciramosum</i> STE-U 972	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. scoparium</i> ATCC 38227	XXXXXXXXXX	XXXXXXCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. scoparium</i> ATCC 46300	CCGAGTTTAC	AAC TCCCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. scoparium</i> STE-U 1720	XXXXXXXXXX	XXXXXXCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
<i>Cy. scoparium</i> STE-U 1722	XXXXXXXXXX	XXXXXXCAA	CCCCATGTGA	ACATACCTGT	TTCGTTCCCT	CGGCGG-XXX
						120
<i>F. subglutinans</i> NRRL 22061	GCCCCGTCCC	GGTAAACCGG	GACGGCCCCG	CAGAGGACCC	C-TAAACTCT	GTT-XXTCTA
<i>Cy. candelabrum</i> STE-U 1674	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. candelabrum</i> STE-U 1677	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. candelabrum</i> STE-U 1951	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. insulare</i> STE-U 616	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. insulare</i> STE-U 768	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. insulare</i> STE-U 954	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. mexicanum</i> STE-U 927	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. mexicanum</i> STE-U 941	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. multiseptatum</i> STE-U 1589	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. multiseptatum</i> STE-U 1602	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. ovatum</i> STE-U 2232	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. ovatum</i> UFV 90	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. pauciramosum</i> STE-U 416	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. pauciramosum</i> STE-U 925	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. pauciramosum</i> STE-U 972	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. scoparium</i> ATCC 38227	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. scoparium</i> ATCC 46300	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. scoparium</i> STE-U 1720	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
<i>Cy. scoparium</i> STE-U 1722	-XXXXTGTCC	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT	TTTGAATTTT
						180
<i>F. subglutinans</i> NRRL 22061	TATGTAACCT	CTGAGTAAAA	CCA-XXTAAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. candelabrum</i> STE-U 1674	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. candelabrum</i> STE-U 1677	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. candelabrum</i> STE-U 1951	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. insulare</i> STE-U 616	TCAGTATCTT	CTGAGTAAAA	AAAA-XCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. insulare</i> STE-U 768	TCAGTATCTT	CTGAGTAAAA	AAAA-XCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. insulare</i> STE-U 954	TCAGTATCTT	CTGAGTAAAA	AAAA-XCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. mexicanum</i> STE-U 927	TCAGTATCTT	CTGAGTAAAA	AAAA-XCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. mexicanum</i> STE-U 941	TCAGTATCTT	CTGAGTAAAA	AAAA-XCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. multiseptatum</i> STE-U 1589	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. multiseptatum</i> STE-U 1602	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. ovatum</i> STE-U 2232	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. ovatum</i> UFV 90	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. pauciramosum</i> STE-U 416	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. pauciramosum</i> STE-U 925	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. pauciramosum</i> STE-U 972	TCAGTATCTT	CTGAGTAAAA	AA-XXXCAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. scoparium</i> ATCC 38227	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. scoparium</i> ATCC 46300	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. scoparium</i> STE-U 1720	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT
<i>Cy. scoparium</i> STE-U 1722	TCAGTATCTT	CTGAGTAAAA	AAAAA-CAA	TAAATCAAAA	CTTTCAACAA	CGGATCTCTT

[illegible][illegible][illegible][illegible]

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<i>F. subglutinans</i> NRRL_22061	GTAAACCCCT CGTTACTGGT AA-TCGTGCG	GGCCACGCCG TTAAC-CCCC	AACTTCTGAA
<i>Cy. candelabrum</i> STE-U_1674	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GACCACGCCG TAAAAACCC	AACTTTTT-X
<i>Cy. candelabrum</i> STE-U_1677	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GACCACGCCG TAAAAACCC	AACTTTTT-X
<i>Cy. candelabrum</i> STE-U_1951	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GACCACGCCG TAAAAACCC	AACTTTTT-X
<i>Cy. insulare</i> STE-U_616	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. insulare</i> STE-U_768	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. insulare</i> STE-U_954	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. mexicanum</i> STE-U_927	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-X
<i>Cy. mexicanum</i> STE-U_941	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-X
<i>Cy. multiseptatum</i> STE-U_1589	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. multiseptatum</i> STE-U_1602	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. ovatum</i> STE-U_2232	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GXXXXXXX XXXXXXXXXX XXXXXXXXXX	XXXXXXXXXX
<i>Cy. ovatum</i> UFV_90	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTTT
<i>Cy. pauciramosum</i> STE-U_416	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-X
<i>Cy. pauciramosum</i> STE-U_925	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-X
<i>Cy. pauciramosum</i> STE-U_972	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-X
<i>Cy. scoparium</i> ATCC_38227	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTTT-
<i>Cy. scoparium</i> ATCC_46300	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. scoparium</i> STE-U_1720	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTT-
<i>Cy. scoparium</i> STE-U_1722	ATACA-XCCT CGCT-CTGGA GTCTCGGTGC	GGCCACGCCG TAAAAACCC	AACTTTTTTT-

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<i>F. subglutinans</i> NRRL_22061	-TGT
<i>Cy. candelabrum</i> STE-U_1674	CTGG
<i>Cy. candelabrum</i> STE-U_1677	CTGG
<i>Cy. candelabrum</i> STE-U_1951	CTGG
<i>Cy. insulare</i> STE-U_616	CTGG
<i>Cy. insulare</i> STE-U_768	CTGG
<i>Cy. insulare</i> STE-U_954	CTGG
<i>Cy. mexicanum</i> STE-U_927	CTGG
<i>Cy. mexicanum</i> STE-U_941	CTGG
<i>Cy. multiseptatum</i> STE-U_1589	CTGG
<i>Cy. multiseptatum</i> STE-U_1602	CTGG
<i>Cy. ovatum</i> STE-U_2232	XXXX
<i>Cy. ovatum</i> UFV_90	CTGG
<i>Cy. pauciramosum</i> STE-U_416	CTGG
<i>Cy. pauciramosum</i> STE-U_925	CTGG
<i>Cy. pauciramosum</i> STE-U_972	CTGG
<i>Cy. scoparium</i> ATCC_38227	CTGG
<i>Cy. scoparium</i> ATCC_46300	CTGG
<i>Cy. scoparium</i> STE-U_1720	CTGG
<i>Cy. scoparium</i> STE-U_1722	CTGG

<i>F. subglutininans</i> NRRL 22061	GCGTTGAGTT	TAT-GGT-XX	XXGCCCTTGA	TTCTACCCCG	C-XXXXTGGG	CGGTGGCAGG
<i>Cy. candelabrum</i> STE-U 1674	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCATCGCT
<i>Cy. candelabrum</i> STE-U 1677	GCG-TGCCTT	TGTTGCT-XX	XXGCCCTTGA	TTCTACCCCG	CGCCCCCGGT	TTCCATCGCT
<i>Cy. candelabrum</i> STE-U 1951	GCG-TGCCTT	TGTTGCT-XX	XXGCCCTTGA	TTCTACCCCG	CGCCCCCGGT	TTCCATCGCT
<i>Cy. insulare</i> STE-U 616	ACG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. insulare</i> STE-U 768	ACG-TGCCTT	TGTTGCT-XX	XXGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. insulare</i> STE-U 954	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. mexicanum</i> STE-U 927	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGG	TTCTACCCCG	CGGTCCCGGT	TTCCACCACC
<i>Cy. mexicanum</i> STE-U 941	GCG-TGCCTT	TGTTGCT-XX	XXGCCCCCTGA	TTCTACCCCG	CGGTCCCGGT	TTCCACCACC
<i>Cy. multiseptatum</i> STE-U 1589	GAG-TGCCTT	TGTTGCTTTC	TGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. multiseptatum</i> STE-U 1602	GAG-TGCCTT	TGTTGCTTTC	TGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. ovatum</i> STE-U 2232	GCG-TGCCTT	TGTTGCT-XX	XXGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. ovatum</i> UFV 90	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. pauciramosum</i> STE-U 416	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. pauciramosum</i> STE-U 925	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. pauciramosum</i> STE-U 972	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. scoparium</i> ATCC 38227	GGG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. scoparium</i> ATCC 46300	GCG-TGCCTT	GTTTGCT-XX	XXGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. scoparium</i> STE-U 1720	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC
<i>Cy. scoparium</i> STE-U 1722	GCG-TGCCTT	TGTTGCT-XX	XGCCCCCTGA	TTCTACCCCG	CGCCCCCGGT	TTCCACCACC

<i>F. subglutininans</i> NRRL 22061	TCAACGACAA	TGCACGAT-X	AG CT-AGCA	GCTTTAA-XA	TACCTTCTGT	CAAGATGAAG
<i>Cy. candelabrum</i> STE-U 1674	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. candelabrum</i> STE-U 1677	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. candelabrum</i> STE-U 1951	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. insulare</i> STE-U 616	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. insulare</i> STE-U 768	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. insulare</i> STE-U 954	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. mexicanum</i> STE-U 927	ACAACGACAA	-CAAAGCCGC	AGCCTCGACA	ACATGAGCAA	GATATCAGGA	TATGATGCT
<i>Cy. mexicanum</i> STE-U 941	ACAACGACAA	-CAAAGCCGC	AGCCTCGACA	ACATGAGCAA	GATATCAGGA	TATGATGCT
<i>Cy. multiseptatum</i> STE-U 1589	CCGACGAAAA	-CAAAGCCGC	AACCTCACGA	ATGTGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. multiseptatum</i> STE-U 1602	CCGACGAAAA	-CAAAGCCGC	AACCTCACGA	ATGTGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. ovatum</i> STE-U 2232	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-CGA	GATATCAGAA	CGAGATGCT
<i>Cy. ovatum</i> UFV 90	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-CGA	GATATCAGAA	CGAGATGCT
<i>Cy. pauciramosum</i> STE-U 416	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. pauciramosum</i> STE-U 925	TGGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. pauciramosum</i> STE-U 972	TCGACGACAA	-CAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCAGAA	CAAGATTGCT
<i>Cy. scoparium</i> ATCC 38227	TCGACGAAAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. scoparium</i> ATCC 46300	TCGACGAAAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. scoparium</i> STE-U 1720	TCGACGAAAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT
<i>Cy. scoparium</i> STE-U 1722	TCGACGAAAA	-CAAAGCCGC	AGCCTCACGA	ACATGA-TGT	GATATCAGAA	CAAGATTGCT

<i>F. subglutininans</i> NRRL 22061	AAGCTAATCA	GATCTTTTCT	CTGCGATAGG	TTCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. candelabrum</i> STE-U 1674	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. candelabrum</i> STE-U 1677	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. candelabrum</i> STE-U 1951	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. insulare</i> STE-U 616	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. insulare</i> STE-U 768	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. insulare</i> STE-U 954	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. mexicanum</i> STE-U 927	AA-C-CGTGT	GCTTCTTTCT	CGACTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. mexicanum</i> STE-U 941	AA-C-CGTGT	GCTTCTTTCT	CGACTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. multiseptatum</i> STE-U 1589	AA-C-CTTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. multiseptatum</i> STE-U 1602	AA-C-CTTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. ovatum</i> STE-U 2232	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. ovatum</i> UFV 90	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. pauciramosum</i> STE-U 416	AA-C-CGTGT	GCTTCTTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. pauciramosum</i> STE-U 925	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. pauciramosum</i> STE-U 972	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. scoparium</i> ATCC 38227	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. scoparium</i> ATCC 46300	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. scoparium</i> STE-U 1720	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG
<i>Cy. scoparium</i> STE-U 1722	AA-C-CGTGT	GCTTCTTTCT	CGATTATAGG	TCCACCTCCA	GACCGGTGAG	TGCGTAAGTG

<i>F. subglutininans</i> NRRL 22061	CTCATCGCTT	CCTCGACGTC	GCATGTGGGG	-GATGCTCAC	-GATGTTT-X	-ATCAGGGTA
<i>Cy. candelabrum</i> STE-U 1674	CCCTTCTCAA	CTCCGACCAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CCATAGGGTA
<i>Cy. candelabrum</i> STE-U 1677	CCCTTCTCAA	CTCCGACCAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CCATAGGGTA
<i>Cy. candelabrum</i> STE-U 1951	CCCTTCTCAA	CTCCGACCAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CCATAGGGTA
<i>Cy. insulare</i> STE-U 616	CTCTTC-XAA	CTCCAACGAA	ATTCTCACGA	CCAGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. insulare</i> STE-U 768	CTCTTC-XAA	CTCCAACGAA	ATTCTCACGA	CCAGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. insulare</i> STE-U 954	CTCTTC-XAA	CTCCAACGAA	ATTCTCACGA	CCAGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. mexicanum</i> STE-U 927	CTCCTTGCAA	CTCCAACAAC	TTTCTCACGG	CCATGATCTC	TGACAGAGCT	CGATAGGGTA
<i>Cy. mexicanum</i> STE-U 941	CTCCTTGCAA	CTCCAACAAC	TTTCTCACGG	CCATGATCTC	TGACAGAGCT	CGATAGGGTA
<i>Cy. multiseptatum</i> STE-U 1589	CTCCTCTCGA	CTCCAACGAT	ATTCTTATGA	CAAGATTAC	TGACAGTTT	CGATAGGGTA
<i>Cy. multiseptatum</i> STE-U 1602	CTCCTCTCGA	CTCCAACGAT	ATTCTTATGA	CAAGATTAC	TGACAGTTT	CGATAGGGTA
<i>Cy. ovatum</i> STE-U 2232	CTTCTCTCGA	CTCCAGCAAG	ATTTTCACGA	CGAGATTGCG	TGACAGTTGT	CAATAGGGTA
<i>Cy. ovatum</i> UFV 90	CTCCTCTCAA	CTCCAACAAG	ATTCTCACGA	CGAGATTGCG	TGACAGTTGT	CGATAGGGTA
<i>Cy. pauciramosum</i> STE-U 416	CTCTTCTCAA	CTCCAACAAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CGATAGGGTA
<i>Cy. pauciramosum</i> STE-U 925	CTCTTCTCAA	CTCCAACAAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CGATAGGGTA
<i>Cy. pauciramosum</i> STE-U 972	CTCTTCTCAA	CTCCAACAAA	ATTCTCACGA	CGAGATTAC	TGACAGTTGT	CGATAGGGTA
<i>Cy. scoparium</i> ATCC 38227	CTCTTC-XAA	CTCCAACAAA	ATTCTCACGA	CCGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. scoparium</i> ATCC 46300	CTCTTC-XAA	CTCCAACAAA	ATTCTCACGA	CCGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. scoparium</i> STE-U 1720	CTCTTC-XAA	CTCCAACAAA	ATTCTCACGA	CCGATTAC	TGACAGTTAT	CGACAGGGTA
<i>Cy. scoparium</i> STE-U 1722	CTCTTC-XAA	CTCCAACAAA	ATTCTCACGA	CCGATTAC	TGACAGTTAT	CGACAGGGTA

F. subglutininans NRRL 22061
Cy. candelabrum STE-U 1674
Cy. candelabrum STE-U 1677
Cy. candelabrum STE-U 1951
Cy. insulare STE-U 616
Cy. insulare STE-U 768
Cy. insulare STE-U 954
Cy. mexicanum STE-U 927
Cy. mexicanum STE-U 941
Cy. multiseptatum STE-U 1589
Cy. multiseptatum STE-U 1602
Cy. ovatum STE-U 2232
Cy. ovatum UVF 90
Cy. pauciramosum STE-U 416
Cy. pauciramosum STE-U 925
Cy. pauciramosum STE-U 972
Cy. scoparium ATCC 38227
Cy. scoparium ATCC 46300
Cy. scoparium STE-U 1720
Cy. scoparium STE-U 1722

[illegible]

F. subglutininans NRRL 22061
Cy. candelabrum STE-U 1674
Cy. candelabrum STE-U 1677
Cy. candelabrum STE-U 1951
Cy. insulare STE-U 616
Cy. insulare STE-U 768
Cy. insulare STE-U 954
Cy. mexicanum STE-U 927
Cy. mexicanum STE-U 941
Cy. multiseptatum STE-U 1589
Cy. multiseptatum STE-U 1602
Cy. ovatum STE-U 2232
Cy. ovatum UVF 90
Cy. pauciramosum STE-U 416
Cy. pauciramosum STE-U 925
Cy. pauciramosum STE-U 972
Cy. scoparium ATCC 38227
Cy. scoparium ATCC 46300
Cy. scoparium STE-U 1720
Cy. scoparium STE-U 1722

[illegible]

F. subglutininans NRRL 22061
Cy. candelabrum STE-U 1674
Cy. candelabrum STE-U 1677
Cy. candelabrum STE-U 1951
Cy. insulare STE-U 616
Cy. insulare STE-U 768
Cy. insulare STE-U 954
Cy. mexicanum STE-U 927
Cy. mexicanum STE-U 941
Cy. multiseptatum STE-U 1589
Cy. multiseptatum STE-U 1602
Cy. ovatum STE-U 2232
Cy. ovatum UVF 90
Cy. pauciramosum STE-U 416
Cy. pauciramosum STE-U 925
Cy. pauciramosum STE-U 972
Cy. scoparium ATCC 38227
Cy. scoparium ATCC 46300
Cy. scoparium STE-U 1720
Cy. scoparium STE-U 1722

TATGCTTTAA	CAGTC AATG	-CCAA -GAAT	TCCCAAGCTC	ACA-XXCAAC	T-XXXXXXXX
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAATCCGAC	TCACAC-XCA
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAATCCGAC	TCACAC-XCA
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAATCCGAC	TCACAC-XCA
TATGTGAAAA	CCACGCGGTG	TACTCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX
TATGTGAAAA	CCACGCGGTG	TACCACACAG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX
TGTGTGAAAA	CCGCGCGGTG	TACTCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX
TATGTAAAAA	CCGCTCCAAG	AAAT'TTCTTT	GTCGGGACGC	CCAAACAAC	TCACA-XXX
TATGTAAAAA	CCGCTCCAAG	AAAT'TTCTTT	GTCGGGACGC	CCAAACAAC	TCACACA-CG
TATGCGAAAA	ATCATGAGTG	CGCTCGCTTT	GTGGAGAATC	ATAGTCAAAC	TGACACACCA
TATGCGAAAA	ATCATGAGTG	CGCTCGCTTT	GTGGAGAATC	ATAGTCAAAC	TGACACACCA
TATGTGAAAA	CCACGCGGAG	CAC'TCCCTTT	ACCGGAAGC	ACAAGCAAAC	TGACACGC-X
TATGTGAAGA	CCACGCGGAG	CACCCCTTTT	GCCGAGAAGC	ACAAGCAAAC	TGACACAC-X
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAAGCCAAAC	TCACAC-XCA
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAAGCCAAAC	TCACACA-XCA
TATGTGAAAA	CCACTCGAAG	CAC'TCCCTTG	ACCGAGAAGC	ACAAGCCAAAC	TCACAC-XCA
TATGTGAAAA	CCACGCGGTG	TACTCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX
TATGTGAAAA	CCACGCGGTG	TTC'TCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAN-XXX
TATGTGAAAA	CCACGCGGTG	TACTCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX
TATGTGAAAA	CCACGCGGTG	TACTCACACG	-CCGAGAGGC	ACAAGCAAAC	TGACAC-XXX

F. *subglutininans* NRRL_22061
Cy. *candelabrum* STE-U_1674
Cy. *candelabrum* STE-U_1677
Cy. *candelabrum* STE-U_1951
Cy. *insulare* STE-U_616
Cy. *insulare* STE-U_768
Cy. *insulare* STE-U_954
Cy. *mexicanum* STE-U_927
Cy. *mexicanum* STE-U_941
Cy. *multiseptatum* STE-U_1589
Cy. *multiseptatum* STE-U_1602
Cy. *ovatum* STE-U_2232
Cy. *ovatum* UVF_90
Cy. *pauciramosum* STE-U_416
Cy. *pauciramosum* STE-U_925
Cy. *pauciramosum* STE-U_972
Cy. *scoparium* ATCC_38227
Cy. *scoparium* ATCC_46300
Cy. *scoparium* STE-U_1720
Cy. *scoparium* STE-U_1722

-XXXXXAGGC	CTCTGGCAAC	AAGTATGTTT	CCCGAGCCGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
T-GTGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
T-GTGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CGTGACGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
TCATGTAGGC	TTCGCGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTTG-TCTGT	CGTGTG-GAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG
-CATGTAGGC	TTCTGGCAAC	AAGTTCGTTT	CTCGCGCTGT	CCTCGTCGAT	CTTGAGCCCG

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<i>F._subglutinans</i> _NRRL_22061	GTACCATGGA	CGCCGTCCGA	GCTGGTCCCT	TCGGTCAGCT	CTTCCGTCCC	GACAACTT
<i>Cy._candelabrum</i> _STE-U_1674	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._candelabrum</i> _STE-U_1677	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._candelabrum</i> _STE-U_1951	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TNGGTCAGNT	CTTTCGCCCC	GACAACTT
<i>Cy._insulare</i> _STE-U_616	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTTCGCCCC	GACAACTT
<i>Cy._insulare</i> _STE-U_768	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTNCGCCCC	GACAACTT
<i>Cy._insulare</i> _STE-U_954	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._mexicanum</i> _STE-U_927	GTACCATGGA	TGCCGTCCGT	GCTGGTCCCT	TCGGTCAGCT	CTTCCGTCCC	GACAACTT
<i>Cy._mexicanum</i> _STE-U_941	GTACCATGGA	TGCCGTCCGT	GCTGGTCCCT	TCGGTCAGCT	CTTCCGTCCC	GACAACTT
<i>Cy._multiseptatum</i> _STE-U_1589	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTTCGCCCC	GACAACTT
<i>Cy._multiseptatum</i> _STE-U_1602	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._ovatum</i> _STE-U_2232	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._ovatum</i> _UFV_90	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._pauciramosum</i> _STE-U_416	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._pauciramosum</i> _STE-U_925	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._pauciramosum</i> _STE-U_972	GTACCATGGA	CGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._scoparium</i> _ATCC_38227	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._scoparium</i> _ATCC_46300	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._scoparium</i> _STE-U_1720	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT
<i>Cy._scoparium</i> _STE-U_1722	GTACCATGGA	TGCCGTCCGT	GCCGGTCCTT	TCGGTCAGCT	CTTCCGCCCC	GACAACTT

Alignment 3. Part 3. Partial *MAT-2* HMG box DNA sequence alignment of selected *Cylindrocladium* species

						60
<i>Cy. multiseptatum</i> STE-U_1602	CCGAAATCAC	GAACAGTGAG	ATTTGTAAGT	ACCCATCGCC	TTATTATAGT	TTCCATGTTA
<i>Cy. multiseptatum</i> STE-U_1589	CGGAAATCAC	GAACAGTGAG	ATTTGTAAGT	ACCCATCGCC	TTATTATAGT	TTCCATGTTA
<i>Cy. pauciramosum</i> STE-U_925	CCGAAATCAC	GAACAGTGAG	ATTTGTAAGT	ACCTACCACC	TTGGCACAAT	TTCTGTGCTG
<i>Cy. pauciramosum</i> STE-U_972	CCGAAATCAC	GAACAGTGAG	ATTTGTAAGT	ACCTACCACC	TTGGCACAAT	TTCTGTGCTG
<i>Cy. candelabrum</i> STE-U_1674	CCGAAATCAC	GAACAGTGAG	ATTTGTAAGT	ACCTACCACC	TTAGCACAAT	TTCTGTACTA
<i>Cy. candelabrum</i> STE-U_1677	CCGAAAACAC	GAACAGTGAG	ATTTGTAAGT	ACCTACCACC	TTAGCACAAT	TTCTGTACTA
<i>Cy. ovatum</i> STE-U_2232	XXXXXXXXXX	XXXXAGTGAG	ATCTGTAAGT	ACCCGCTACC	CTAGCACAGT	TTCTGTACTA
<i>Cy. insulare</i> STE-U_616	CAGAAATTAC	CAACAGTGAG	ATTTGTAGGT	ACTCACCACC	TTGGTACAGT	TTCTGTACTA
<i>Cy. insulare</i> STE-U_768	CAGAAATCAC	CAACAGTGAG	ATTTGTAAGT	ACTCACCACC	TTGGTACAGT	TTCTGTACTA
<i>Cy. scoparium</i> STE-U_1720	XXXXXXXXXX	XAACAGTGAG	ATTTGTAAGT	ACTCACCACC	TTGGTACAGT	TTCTGTACTG
<i>Cy. scoparium</i> ATCC_38227	XXXXXXXXXAC	CAACAGTGAG	ATTTGTAAGT	ACTCACCACC	TTGGTACAGT	TTCTATACTA
						120
<i>Cy. multiseptatum</i> STE-U_1602	ACACTTTTCA	GCCATGGTTC	TTGGTCGCGC	CTGGAACATG	GAGACTCCGG	AGACGCGCAA
<i>Cy. multiseptatum</i> STE-U_1589	ACACTTTTCA	GCCATGGTTC	TTGGTCGCGC	CTGGAACATG	GAGACTCCGG	AGACGCGCAA
<i>Cy. pauciramosum</i> STE-U_925	ACATTTTTC	GCCATGGTTC	TTGGCCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. pauciramosum</i> STE-U_972	ACATTTTTC	GCCATGGTTC	TTGGCCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. candelabrum</i> STE-U_1674	AAAGTTTTC	GCCATGGTTC	TTGGCCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. candelabrum</i> STE-U_1677	AAAGTTTTC	GCCATGGTTC	TTGGCCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. ovatum</i> STE-U_2232	ACATTTTTC	GCTATGGTTC	TGGGTCGTGC	CTGGAACATG	GAAACTCCAG	AAACGCGAAA
<i>Cy. insulare</i> STE-U_616	ACATTTTTC	GCCATGGTTC	TTGGTCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. insulare</i> STE-U_768	ACATTTTTC	GCCATGGTTC	TTGGTCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. scoparium</i> STE-U_1720	ACATTTTTC	GCCATGGTTC	TTGGTCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
<i>Cy. scoparium</i> ATCC_38227	ACATTTTTC	GCCATGGTTC	TTGGTCGTGC	CTGGAACATG	GAGACTCCAG	AGACGCGAAA
						171
<i>Cy. multiseptatum</i> STE-U_1602	GAAGTATAAG	CTCATGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. multiseptatum</i> STE-U_1589	GAAGTATAAG	CTCATGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. pauciramosum</i> STE-U_925	GAAGTACAAG	CTCAAGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. pauciramosum</i> STE-U_972	GAAGTACAAG	CTCAAGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. candelabrum</i> STE-U_1674	GAAGTACAAG	CTCAAGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. candelabrum</i> STE-U_1677	GAAGTACAAG	CTCAAGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. ovatum</i> STE-U_2232	GAAGTACAAA	CTCATGGCAG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. insulare</i> STE-U_616	GAAGTACAAG	CTCATGGCGG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. insulare</i> STE-U_768	GAAGTACAAG	CTCATGGCGG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. scoparium</i> STE-U_1720	GAAGTACAAG	CTCATGGCGG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A
<i>Cy. scoparium</i> ATCC_38227	GAAGTACAAG	CTCATGGCGG	ATGAGATCAA	GGCTGAGCTC	ATCAAGAAGC	A

Alignment 4. Part 4. 5' end of β -tubulin gene DNA sequence alignment of *Cylindrocladium**pauciramosum*

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<i>F. subglutinans</i> _NRRL_22061	GCGTTGAGTT	TATGG-T-XX	XXXGCCCTG	ATTCTACCCC	GCTGGGC-GG	TGGC-AGCTC
<i>Cy. candelabrum</i> _STE-U_1674	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCATCGC
<i>Cy. candelabrum</i> _STE-U_1677	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCATCGC
<i>Cy. candelabrum</i> _STE-U_1951	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCATCGC
<i>Cy. candelabrum</i> _UFV_89	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCATCGC
<i>Cy. mexicanum</i> _STE-U_927	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	GTTCTACCCC	GCCGTCCCGG	TTTCCACCGC
<i>Cy. mexicanum</i> _STE-U_941	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGTCCCGG	TTTCCACCGC
<i>Cy. multiseptatum</i> _STE-U_1589	GAG-TGCCTT	TGTTGCTTT-	CTGGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. multiseptatum</i> _STE-U_1602	GAG-TGCCTT	TGTTGCTTTG	CT-GCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_127	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_128	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_192	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_196	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_2	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_26	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_6	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_60	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_62	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _DSTEF_84	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_1160	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_143	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_1691	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_1692	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_1990	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_2030	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_344	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_416	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_913	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_925	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_951	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_971	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _STE-U_972	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _UFV_25	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC
<i>Cy. pauciramosum</i> _UFV_27	GCG-TGCCTT	TGTTGCT-XX	XXXGCCCTG	ATTCTACCCC	GCCGCCCCGG	TTTCCACCGC

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<i>F. subglutinans</i> _NRRL_22061	AACGACAATG	-CACGATAGC	-TAGCAGCTT	TAAATACC-T	TCTGTCAAGA	TGAAGAA-GC
<i>Cy. candelabrum</i> _STE-U_1674	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. candelabrum</i> _STE-U_1677	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. candelabrum</i> _STE-U_1951	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. candelabrum</i> _UFV_89	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. mexicanum</i> _STE-U_927	TACACGACA	ACAAAGCCGC	AGCCTCGACA	ACATGAGCAA	GATATCA-GG	ATATGATGGC
<i>Cy. mexicanum</i> _STE-U_941	TACACGACA	ACAAAGCCGC	AGCCTCGACA	ACATGAGCAA	GATATCA-GG	ATATGATGGC
<i>Cy. multiseptatum</i> _STE-U_1589	TCCGACGAAA	ACAAAGCCGC	AACCTCACGA	ATGTGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. multiseptatum</i> _STE-U_1602	TCCGACGAAA	ACAAAGCCGC	AACCTCACGA	ATGTGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_127	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_128	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_192	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_196	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_2	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_26	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_6	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_60	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_62	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _DSTEF_84	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1160	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_143	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1670	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1671	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1691	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1692	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_1990	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_2030	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_344	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_416	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_913	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_925	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_951	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATGA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_971	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC
<i>Cy. pauciramosum</i> _STE-U_972	TTTCGACGACA	ACAAAGCCGC	AGCCTCACGA	TCATAA-CGA	GATATCA-GA	ACAAGATTGC

[illegible][illegible]

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<i>F. subglutinans</i> _NRRL_22061	GGTACCATGG	ACGCCGTCCG	AGCTGGTCCC	TTCGGTCAGC	T
<i>Cy. mexicanum</i> _STE-U_927	GGTACCATGG	ATGCCGTCCG	TGCTGGTCCC	TTCGGTCAGC	T
<i>Cy. mexicanum</i> _STE-U_941	GGTACCATGG	ATGCCGTCCG	TGCTGGTCCC	TTCGGTCAGC	T
<i>Cy. multiseptatum</i> _STE-U_1602	GGTACCATGG	ATGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. multiseptatum</i> _STE-U_1589	GGTACCATGG	ATGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. candelabrum</i> _STE-U_1951	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. candelabrum</i> _STE-U_1674	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. candelabrum</i> _UFV_89	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. candelabrum</i> _STE-U_1677	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1671	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1670	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_416	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_972	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_143	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_2	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_127	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_128	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_84	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_2030	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_925	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_913	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1160	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_951	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1990	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1692	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_344	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_971	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _STE-U_1691	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_60	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_62	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_6	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_26	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_196	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T
<i>Cy. pauciramosum</i> _DISTEF_192	GGTACCATGG	ACGCCGTCCG	TGCCGGTCCT	TTCGGTCAGC	T

Alignment 5. Part 5. 5' end of β -tubulin gene DNA sequence alignment from *Cylindrocylindrium* species

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<i>F. subglutinans</i> NRRL_22061	GCGTT-GAGT	TTATG-XXXX	XGT-GCCCCCT	GATTCTACCC	CGCTGGGG-G	-XXXXXGTGG-
<i>Cy. avesciculatum</i> ATCC_38226	GCGTGCC-TT	TGTTG-XXXX	XCC-GCCCCCT	GATTCTACCC	CGCCGTCCCG	-XXXXXGTTTC
<i>Cy. candelabrum</i> STE-U_1674	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. candelabrum</i> STE-U_1677	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. candelabrum</i> STE-U_1951	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. candelabrum</i> UFV_89	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. citri</i> CBS_186.36	GCGTGCC-TT	TGTTGTTGTT	GCT-GCCCCCT	GATCCTACCC	CGCCGCCCCA	TGGGTGTTTC
<i>Cy. colhounii</i> STE-U_1237	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. colhounii</i> STE-U_1339	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. colhounii</i> STE-U_681	GCGTGCC-TT	TGTTG-XXXX	XCT-GACCCT	GATTCTACCC	CGACGACCCG	-XXXXXGTTTC
<i>Cy. colhounii</i> STE-U_705	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. curvisporum</i> STE-U_763	GCGTGCC-TT	TGTT-XXXXX	-CT-GCCCCCT	GATTCTACCC	CCCCGCCCCG	-XXXXXGTTTC
<i>Cy. curvisporum</i> STE-U_765	GCGTGCC-TT	TGTT-XXXXX	-CT-GCCCCCT	GAGCGTACCC	CCCCGCCCCG	-XXXXXGTTTC
<i>Cy. flexuosum</i> STE-U_2536	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> ATCC_18834	GCGTGCC-TT	TGTTA-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> ATCC_18882	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> CBS_413.67	XXXXXGCC-TT	TGTTG-XXXX	GCT-GCCCCCT	GATTGTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> IMI_35428	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> IMI_35429	XXXXXGCC-TT	GTTTG-XXXX	XCT-GCCCCA	GAACGTACCA	CGCCGCCCTG	-XXXXXGTTAC
<i>Cy. floridanum</i> STE-U_2350	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> STE-U_682	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. floridanum</i> UFV_76	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. gracile</i> ATCC_22833	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. gracile</i> IMI_167580	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. gracile</i> PC_551197	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. gracile</i> STE-U_1586	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. gracile</i> STE-U_623	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. graciloides</i> STE-U_1153	ACGTGCC-TT	GGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. hawksworthii</i> MJCL_30866	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. heptaseptatum</i> FTCC_1002	GCGGCGN-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. heptaseptatum</i> FTCC_1003	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATACTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. heptaseptatum</i> STE-U_2344	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. insulare</i> STE-U_616	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. insulare</i> STE-U_768	ACGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. insulare</i> STE-U_954	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. leucothoes</i> ATCC_64824	XXXXXXXXXX	XXXXXXXXXX	XCT-GCCCCCT	GATCCTACCC	CGCCGCCCTG	GGGG-ATTTT
<i>Cy. leucothoes</i> P97_2605	GCGAGCC-TT	TGTTGTTGTT	GCT-GCCCCCT	GATCCTACCC	CGCCGCCCTG	GGGG-ATTTT
<i>Cy. macroconidiale</i> STE-U_307	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. macroconidiale</i> STE-U_413	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. mexicanum</i> STE-U_927	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GGTTCTACCC	CGCCGTCCTG	-XXXXXGTTTC
<i>Cy. mexicanum</i> STE-U_941	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGTCCTG	-XXXXXGTTTC
<i>Cy. multiseptatum</i> STE-U_1589	GAGTGCC-TT	TGTTGCT-TT	-CTGGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. multiseptatum</i> STE-U_1602	GAGTGCC-TT	TGTTGCT-TT	GCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. naviculatum</i> STE-U_627	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXAAATTC
<i>Cy. naviculatum</i> STE-U_628	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXAAATTC
<i>Cy. ovatum</i> UFV_90	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. parasiticum</i> ATCC_46133	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGCCCYG	-XXXXXGTTTC
<i>Cy. parasiticum</i> CBS_190.50	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. parasiticum</i> STE-U_723	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GAGCGTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pauciramosum</i> STE-U_416	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pauciramosum</i> STE-U_925	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pauciramosum</i> STE-U_972	GCGTGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. penicilloides</i> CBS_174.55	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pseudogracile</i> AR_2677	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pseudogracile</i> STE-U_1588	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pteridis</i> STE-U_2869	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pteridis</i> STE-U_2190	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. pteridis</i> UFV_43	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. quinqueseptatum</i> ATCC_16550	GCGTGCC-TT	GGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. quinqueseptatum</i> STE-U_516	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. quinqueseptatum</i> STE-U_759	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. rumohrae</i> STE-U_1603	XXXXXGCC-TT	TGTTG-XXXX	XCTTGCCCCCT	AATTCAACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. rumohrae</i> UFV_215	GCGTGCCCTT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. rumohrae</i> UFV_218	GCGTGCCCTT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. scoparium</i> ATCC_38227	GGGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. scoparium</i> ATCC_46300	GCGTGCC-TT	GGTTG-XXXX	XCTTGCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. scoparium</i> STE-U_1720	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. scoparium</i> STE-U_1722	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_599	XXXXXXC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCYG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_1150	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_1484	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_2321	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_2322	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGACCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_2347	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGATCCG	-XXXXXGTTTC
<i>Cy. sp.</i> STE-U_2712	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathiphylli</i> ATCC_44730	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathiphylli</i> STE-U_1624	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathiphylli</i> STE-U_1641	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathiphylli</i> STE-U_2186	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathiphylli</i> STE-U_2188	XXXXXGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathulatum</i> AR_1844	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. spathulatum</i> ATCC_62616	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. theae</i> ATCC_48895	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. theae</i> UFV_16A	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. variabile</i> AR_2675	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC
<i>Cy. variabile</i> UFV_28	GCGTGCC-TT	TGTTG-XXXX	XCT-GCCCCCT	GATTCTACCC	CGCCGCCCCG	-XXXXXGTTTC

[illegible]

[illegible]

F. subglutinans NRRL 22061 ACCGGTCAGT GCGTAAGTGC TCATCG-CTT CC-TCGAC-X -XXGTCGCAT -XGTGGGG-G
 Cy. avesculatum ATCC 38226 ACCGGTCAGT GCGTAAGTGC TCTCAT-CAA CC-CCGAAA AA-AACCTTTCT -CGAGGCCAT
 Cy. candelabrum STE-U 1674 ACCGGTCAGT GCGTAAGTAC CCTTCT-CAA CT-CCGACCA AA-XXXTTCT -CACGACGAG
 Cy. candelabrum STE-U 1677 ACCGGTCAGT GCGTAAGTAC CCTTCT-CAA CT-CCGACCA AA-XXXTTCT -CACGACGAG
 Cy. candelabrum STE-U 1951 ACCGGTCAGT GCGTAAGTAC CCTTCT-CAA CT-CCGACCA AA-XXXTTCT -CACGACGAG
 Cy. candelabrum UFV 89 ACCGGTCAGT GCGTAAGTAC CCTTCT-CAA CT-CCGACCA AA-XXXTTCT -CACGACGAG
 Cy. citri CBS 185.36 ACCGGTCAGT GCGTAAGTGC CCATCTTCAA CT-CCGAAA AA-XCTTTCT -CACGGCAT
 Cy. colhounii STE-U 1237 ACCGGTCAGT GCGTAAGTGC TCTTGT-CAA CT-CCAACAA TA-XXXXTTAT -CAC-XXGAG
 Cy. colhounii STE-U 1339 ACCGGTCAGT GCGTAAGTGC TCTTGT-CAA CT-CCAACAA TA-XXXXTTAT -CAC-XXGAG
 Cy. colhounii STE-U 681 ACCGGTCAGT GCGTAAGTGC TCTTGT-CAA CT-CCAACAA TA-XXXXTTAT -CAC-XXGAG
 Cy. colhounii STE-U 705 ACCGGTCAGT GCGTAAGTGC TCTTGT-CAA CT-CCAACAA TA-XXXXTTAT -CAC-XXGAG
 Cy. curvisporum STE-U 763 ACCGGTCAGT GCGTAAGTGA TCATTC-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. curvisporum STE-U 765 ACCGGTCAGT GCGTAAGTGA TCATTC-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. flexuosum STE-U 2536 ACCGGTCAGT GCGTAAGTGA TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CATGACGA
 Cy. floridanum ATCC 18834 ACCGGTCAGT GCGTAAGTGA TAGTTCCTCAA CTT-CAAAA AAAA-XTCT -ACCGTGAAG
 Cy. floridanum ATCC 18882 ACCGGTCAGT GCGTAAGTGA TAGTTCCTCAA CTT-CAAAA AAAA-XTCT -ACCGTGAAG
 Cy. floridanum CBS 413.67 ACCGGTCAGT GCGTAAGTGT TCATTCCGAA T-CCAAG- XXXXXXTCT -GCCCGGAG
 Cy. floridanum IMI 35428 ACCGGTCAGT GCGTAAGTGA TCATT-CCAG CTTCAA-AA A-XXXXXXCT -CCCCTGAGG
 Cy. floridanum IMI 35429 ACCGGTCAAC GCGTAAGTGA TAATTT-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. floridanum STE-U 2350 ACCGGTCAGT GCGTAAGTGA TAGTTCCTCAA CTTCAAAAA AAAA-XTCT -ACCGTGAAG
 Cy. floridanum STE-U 682 ACCGGTCAGT GCGTAAGTGA TAGTTCCTCAA CTTCAAAAA AAAA-XTCT -ACCGNAGG
 Cy. floridanum UFV 76 ACCGGTCAGT GCGTAAGTGA TTATT-CCAG CTTCAA-AA A-XXXXXXCT -GCCTTGGGG
 Cy. gracile ATCC 22833 ACCGGTCAGT GCGTAAGTGA TATTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGATGAG
 Cy. gracile IMI 167580 ACCGGTCAGT GCGTAAGTGA TATTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. gracile PC 551197 ACCGGTCAGT GCGTAAGTGA TATTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. gracile STE-U 1586 ACCGGTCAGT GCGTAAGTGA TATTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. gracile STE-U 623 ACCGGTCAGT GCGTAAGTGA TATTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGATGAG
 Cy. graciloides STE-U 1153 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CGCGGCCA
 Cy. hawksworthii MUC 30866 ACCGGTCAGT GCGTAAGTGC TCTT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. heptaseptatum FTCC 1002 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CCCCTGAGG
 Cy. heptaseptatum FTCC 1003 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CGCGGCCA
 Cy. heptaseptatum STE-U 2344 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CCCCTGAGG
 Cy. insulare STE-U 616 ACCGGTCAGT GCGTAAGTGC TCTT-XXCAA CT-CCAACGG AA-XXXXTTCT -CACGACGAG
 Cy. insulare STE-U 768 ACCGGTCAGT GCGTAAGTGC TCTT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. insulare STE-U 954 ACCGGTCAGT GCGTAAGTGC TCTT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. leucothoes ATCC 64824 ACCGGTCAGT GCGTAAGTGA TCCTATTCAA CCCCCA-AA A-XXCTTTCT -CGCGGCCAT
 Cy. leucothoes P97.2605 ACCGGTCAGT GCGTAAGTGA TCCTATTCAA CCCCCA-AA A-XXCTTTCT -CGCGGCCAT
 Cy. macroconidiale STE-U 307 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCGACAA TA-XXXXTTAT -CACGGCGAG
 Cy. macroconidiale STE-U 413 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCGACAA TA-XXXXTTAT -CACGGCGAG
 Cy. mexicanum STE-U 927 ACCGGTCAGT GCGTAAGTAT TCCTTG-CAA CT-CCAACAA -XXXXTTCT -CACGGCCAT
 Cy. mexicanum STE-U 941 ACCGGTCAGT GCGTAAGTAT TCCTTG-CAA CT-CCAACAA -XXXXTTCT -CACGGCCAT
 Cy. multiseptatum STE-U 1589 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAACGA TA-XXXXTTCT -TATGACAAG
 Cy. multiseptatum STE-U 1602 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAACGA TA-XXXXTTCT -TATGACAAG
 Cy. naviculatum STE-U 627 ACCGGTCAGT GCGTAAGT-A TTTAATCGA CT-CCAG-AA -XXXXXTTCG TCGTGATGAG
 Cy. naviculatum STE-U 628 ACCGGTCAGT GCGTAAGT-A TTTAATCGA CT-CCAG-AA -XXXXXTTCG TCGTGATGAG
 Cy. ovatum UFV 90 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAACAA GA-XXXXTTCT -CACGACGAG
 Cy. parasiticum ATCC 46133 ACCGGTCAGT GCGTAAGTGA TCATTC-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. parasiticum CBS 190.50 ACCGGTCAGT GCGTAAGTGA TCATTC-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. parasiticum STE-U 723 ACCGGTCAGT GCGTAAGTGA TCATTC-CAG CTTCAA-AA A-XXXXXXCT -GCCCTGAGG
 Cy. pauciramosum STE-U 416 ACCGGTCAGT GCGTAAGTAT TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. pauciramosum STE-U 925 ACCGGTCAGT GCGTAAGTAT TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. pauciramosum STE-U 972 ACCGGTCAGT GCGTAAGTAT TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. penicillioideus CBS 174.55 ACCGGTCAGT GCGTAAGTAT TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. pseudogracile AR 2677 ACCGGTCAGT GCGTAAGTGA TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. pseudogracile STE-U 1588 ACCGGTCAGT GCGTAAGTGA TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. pteridis STE-U 2869 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAGCAA GA-XXXXTTT -CACGACGAG
 Cy. pteridis STE-U 2190 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAGCAA GA-XXXXTTT -CACGACGAG
 Cy. pteridis UFV 43 ACCGGTCAGT GCGTAAGTGC TCCTCT-CAA CT-CCAGCAA GA-XXXXTTT -CACGACGAG
 Cy. quinquesepalum ATCC 16550 ACCGGTCAGT GCGTAAGTGC TCCTCT-XXG CT-CCGAAA TG-XXXXTTCT -CATGACAAC
 Cy. quinquesepalum STE-U 516 ACCGGTCAGT GCGTAAGTGC TCCTCT-XXG CT-CCGAAA TG-XXXXTTCT -CATGACAAC
 Cy. quinquesepalum STE-U 759 ACCGGTCAGT GCGTAAGTGC TCCTCT-XXG CT-CCGAAA TG-XXXXTTCT -CATGACAAC
 Cy. rumohrae STE-U 1603 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CGCAGCGG
 Cy. rumohrae UFV 215 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CGCAGCGG
 Cy. rumohrae UFV 218 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CGCAGCGG
 Cy. scoparium ATCC 38227 ACCGGTCAGT GCGTAAGTGC TATTCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. scoparium ATCC 46300 ACCGGTCAGT GCGTAAGTGC TATTCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. scoparium STE-U 1720 ACCGGTCAGT GCGTAAGTGC TATTCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. scoparium STE-U 1722 ACCGGTCAGT GCGTAAGTGC TATTCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. sp. STE-U 599 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 1150 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 1484 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 2321 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 2322 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 2347 ACCGGTCAGT GCGTAAGTGC TCTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. sp. STE-U 2712 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. spathiphylli ATCC 44730 ACCGGTCAGT GCGTAAGTGC TCCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. spathiphylli STE-U 1624 ACCGGTCAGT GCGTAAGTGC TACT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. spathiphylli STE-U 1641 ACCGGTCAGT GCGTAAGTGC TACT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. spathiphylli STE-U 2186 ACCGGTCAGT GCGTAAGTGC TCCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. spathiphylli STE-U 2188 ACCGGTCAGT GCGTAAGTGC TCCT-XXCAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. spathulatum AR 1844 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. spathulatum ATCC 62616 ACCGGTCAGT GCGTAAGTAT TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACGAG
 Cy. theae ATCC 48895 ACCGGTCAGT GCGTAAGTGC TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG
 Cy. theae UFV 16A ACCGGTCAGT GCGTAAGTGC TTTTCT-CAA CT-CCAACAA AA-XXXXTTCT -CACGACCGG

<i>F. subglutinans</i> _NRRL_22061	A-XXXTGCTC	AC-XGAGTTT	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAAACCAT
<i>Cy. avesiculatum</i> _ATCC_38226	ATT-XCACTG	ACAGGAGTCG	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. candelabrum</i> _STE-U_1674	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. candelabrum</i> _STE-U_1677	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. candelabrum</i> _STE-U_1951	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. candelabrum</i> _UFV_89	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. citri</i> _CBS_186.36	GTT-XCTCTA	ACTCTCGTCG	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. colhounii</i> _STE-U_1237	ATT-XTACTG	ACATATGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. colhounii</i> _STE-U_1339	ATT-XTACTG	ACACATGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. colhounii</i> _STE-U_681	ATT-XTACTG	ACATATGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. colhounii</i> _STE-U_705	ATT-XTACTG	ACATATGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. curvisporum</i> _STE-U_763	ATT-XCACTA	ACA-TTCGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. curvisporum</i> _STE-U_765	ATT-XCACTA	ACA-TTCGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. flexuosum</i> _STE-U_2536	ATT-XTACTG	ACGTTGTGCG	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _ATCC_18834	ATT-XGACTG	ACACTTATGG	CT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _ATCC_18882	ATT-XGACTG	ACACTTATGG	CT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _CBS_413.67	ATT-XCACTG	ACATTTGTCC	TT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCANACCAT
<i>Cy. floridanum</i> _IMI_35428	ATT-XCACTA	ACA-TTCGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _IMI_35429	ATT-XCACTA	ACA-TTCGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAAACCAT
<i>Cy. floridanum</i> _STE-U_2350	ATT-XGACTG	ACACTTATGG	CT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _STE-U_682	ATT-XGACTG	ACACTTATGG	CT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. floridanum</i> _UFV_76	ATT-XCACTA	ACA-TTCGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. gracile</i> _ATCC_22833	ATT-XCACTG	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. gracile</i> _IMI_167580	ATT-XCACTG	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. gracile</i> _PC_551197	ATT-XCACTG	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. gracile</i> _STE-U_1586	ATT-XCACTG	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. gracile</i> _STE-U_623	ATT-XCACTG	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. graciloides</i> _STE-U_1153	GATT-CGCTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. hawksworthii</i> _MUC_30866	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. heptaseptatum</i> _FTCC_1002	ATT-XTACTG	ACATGTTTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. heptaseptatum</i> _FTCC_1003	ATT-XTACTG	ACATGTTTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. heptaseptatum</i> _STE-U_2344	ATT-XTACTG	ACATGTTTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. insulare</i> _STE-U_616	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. insulare</i> _STE-U_768	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. insulare</i> _STE-U_954	ATT-XCACTG	ACAAATTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. leucothoes</i> _ATCC_64824	GTT-XCACTG	ACAGTCGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. leucothoes</i> _P97.2605	GTT-XCACTG	ACAGTCGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. macroconidiale</i> _STE-U_307	GTT-XTACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. macroconidiale</i> _STE-U_413	GTT-XTACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. mexicanum</i> _STE-U_927	GAT-XCGCTG	ACAGACGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. mexicanum</i> _STE-U_941	GAT-XCGCTG	ACAGACGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. multiseptatum</i> _STE-U_1589	ATT-XCACTG	ACAGTTTTCG	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. multiseptatum</i> _STE-U_1602	ATT-XCACTG	ACAGTTTTCG	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. naviculatum</i> _STE-U_627	ATT-XTGCTG	AC-CTTGGTG	GACAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. naviculatum</i> _STE-U_628	ATT-XTGCTG	AC-CTTGGTG	GACAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. ovatum</i> _UFV_90	ATT-XCGCTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. parasiticum</i> _ATCC_46133	ATT-XCACTA	ACA-TTSGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. parasiticum</i> _CBS_190.50	ATT-XCACTA	ACA-TTSGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. parasiticum</i> _STE-U_723	ATT-XCACTA	ACA-TTSGCG	ATCAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pauciramosum</i> _STE-U_416	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pauciramosum</i> _STE-U_925	GTT-XCGCTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pauciramosum</i> _STE-U_972	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. penicilloides</i> _CBS_174.55	ATT-XCGCTC	ACAAAT-CACA	AACAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAAACCAT
<i>Cy. pseudogracile</i> _AR_2677	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pseudogracile</i> _STE-U_1588	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pteridis</i> _STE-U_2869	ATT-XCGCTG	ACAGTTGTCA	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pteridis</i> _STE-U_2190	ATT-XCGCTG	ACAGTTGTCA	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. pteridis</i> _UFV_43	ATT-XCGCTG	ACAGTTGTCA	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. quinqueseptatum</i> _ATCC_16550	GTT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. quinqueseptatum</i> _STE-U_516	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. quinqueseptatum</i> _STE-U_759	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. rumohrae</i> _STE-U_1603	ATT-XAAGT	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. rumohrae</i> _UFV_215	ATA-XAAGT	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. rumohrae</i> _UFV_218	ATA-XAAGT	ACATTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. scoparium</i> _ATCC_38227	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. scoparium</i> _ATCC_46300	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. scoparium</i> _STE-U_1720	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. scoparium</i> _STE-U_1722	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_599	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_1150	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_1484	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_2321	ATT-XTACTG	ACAGCTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_2322	ATT-XTACTG	ACAGCTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_2347	ATT-XTACTG	ACAGCTGTCC	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. sp.</i> _STE-U_2712	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathiphylli</i> _ATCC_44730	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathiphylli</i> _STE-U_1624	ATT-XTACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathiphylli</i> _STE-U_1641	ATT-XTACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathiphylli</i> _STE-U_2186	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathiphylli</i> _STE-U_2188	ATT-XCACTG	ACAGTTATCG	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathulatum</i> _AR_1844	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. spathulatum</i> _ATCC_62616	ATT-XCACTG	ACAGTTGTCC	AT-AGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. theae</i> _ATCC_48895	ATT-XCGCTG	ACACTCGCGG	AT-AGGGTAA	CCAAATCGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. theae</i> _UFV_16A	ATT-XCGCTG	ACACTCGCGG	AT-AGGGTAA	CCAAATCGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. variabile</i> _AR_2675	ATT-XCACTG	ACAGTTATCA	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT
<i>Cy. variabile</i> _UFV_28	ATT-XCACTG	ACAGTTATCA	A-CAGGGTAA	CCAAATTGGT	GCTGCTTTCT	GGCAGACCAT

<i>F. subglutinans</i> NRRL 22061	CTCTGGCGAG	CACGGCTCG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. avesiculatum</i> ATCC 38226	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. candelabrum</i> STE-U 1674	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. candelabrum</i> STE-U 1677	CTSTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. candelabrum</i> STE-U 1951	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. candelabrum</i> UFV 89	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. citri</i> CBS 186.36	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. colhounii</i> STE-U 1237	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. colhounii</i> STE-U 1339	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. colhounii</i> STE-U 681	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. colhounii</i> STE-U 705	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. curvisporum</i> STE-U 763	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. curvisporum</i> STE-U 765	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. flexuosum</i> STE-U 2536	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. floridanum</i> ATCC 18834	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. floridanum</i> ATCC 18882	TTTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	AGCTCCAGTT
<i>Cy. floridanum</i> CBS 413.67	CTTTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. floridanum</i> IMI 35428	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTTTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. floridanum</i> IMI 35429	CTTTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	AGCTCCAGTT
<i>Cy. floridanum</i> STE-U 2350	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. floridanum</i> STE-U 682	CTTTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	ACCTCCAGTT
<i>Cy. floridanum</i> UFV 76	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACAAC	GGTACCTCCG	AGCTCCAGTT
<i>Cy. gracile</i> ATCC 22833	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. gracile</i> IMI 167580	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. gracile</i> PC 551197	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. gracile</i> STE-U 1586	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. gracile</i> STE-U 623	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. graciloidum</i> STE-U 1153	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. hawksworthii</i> MUCCL 30866	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. heptaseptatum</i> FTCC 1002	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTNTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. heptaseptatum</i> FTCC 1003	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. heptaseptatum</i> STE-U 2344	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTTTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. insulare</i> STE-U 616	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. insulare</i> STE-U 768	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. insulare</i> STE-U 954	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. leucothoes</i> ATCC 64824	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. leucothoes</i> P97.2605	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. macroconidiale</i> STE-U 307	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. macroconidiale</i> STE-U 413	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. mexicanum</i> STE-U 927	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. mexicanum</i> STE-U 941	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. multiseptatum</i> STE-U 1589	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. multiseptatum</i> STE-U 1602	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. naviculatum</i> STE-U 627	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGCACCTCTG	AGCTCCAGCT
<i>Cy. naviculatum</i> STE-U 628	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACAAC	GGCACCTCTG	AGCTCCAGCT
<i>Cy. ovatum</i> UFV 90	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. parasiticum</i> ATCC 46133	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. parasiticum</i> CBS 190.50	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. parasiticum</i> STE-U 723	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pauciramosum</i> STE-U 416	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pauciramosum</i> STE-U 925	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pauciramosum</i> STE-U 972	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. penicilloides</i> CBS 174.55	CTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	ATCTCCAGCT
<i>Cy. pseudogracile</i> AR 2677	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AACTCCAGCT
<i>Cy. pseudogracile</i> STE-U 1588	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pteridis</i> STE-U 2869	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pteridis</i> STE-U 2190	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. pteridis</i> UFV 43	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. quinqueseptatum</i> ATCC 16550	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. quinqueseptatum</i> STE-U 516	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. quinqueseptatum</i> STE-U 759	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. rumohrae</i> STE-U 1603	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. rumohrae</i> UFV 215	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. rumohrae</i> UFV 218	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. scoparium</i> ATCC 38227	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. scoparium</i> ATCC 46300	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. scoparium</i> STE-U 1720	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. scoparium</i> STE-U 1722	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 599	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 1150	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 1484	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 2321	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 2322	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 2347	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. sp.</i> STE-U 2712	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathiphylli</i> ATCC 44730	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathiphylli</i> STE-U 1624	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathiphylli</i> STE-U 1641	CTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathiphylli</i> STE-U 2186	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathiphylli</i> STE-U 2188	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathulatum</i> AR 1844	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. spathulatum</i> ATCC 62616	TTCTGGCGAG	CACGGCTCTG	ACAGCAATGG	CGTCTACGCC	GGTACCTCCG	AGCTCCAGCT
<i>Cy. theae</i> ATCC 48895	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. theae</i> UFV 16A	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. variabile</i> AR 2675	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT
<i>Cy. variabile</i> UFV 28	TTCTGGCGAG	CACGGTCTCG	ACAGCAATGG	TGTCTACGCT	GGTACCTCCG	AGCTCCAGCT

<i>F. subglutinis</i> NRRL 22061	CGAGCGTATG	ACTGCTTACT	TCAACGAGGT	ATGC-TTTAA	-XCA-G-TCA	ATGCCAA-GA
<i>Cy. avesciculatum</i> ATCC 38226	CGAGCGTATG	AACGCTTACT	TTCACGAGGT	GTGTA-XAAA	ACCGCGCCGA	-GAACTTTC-
<i>Cy. candelabrum</i> STE-U 1674	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACTC-GA	AGCACTCCC-
<i>Cy. candelabrum</i> STE-U 1677	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACTC-GA	AGCACTCCC-
<i>Cy. candelabrum</i> STE-U 1951	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACTC-GA	AGCACTCCC-
<i>Cy. candelabrum</i> UFV 89	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACTC-GA	AGCACTCCC-
<i>Cy. citri</i> CBS 186.36	CGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCGCGCCGA	-GAACCTCC-
<i>Cy. colhounii</i> STE-U 1237	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	GCTGC-TTGG	TGT-XCCCC-
<i>Cy. colhounii</i> STE-U 1339	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	GCTGC-TTGG	TGTA-CCCC-
<i>Cy. colhounii</i> STE-U 681	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	GCTGC-TTGG	TGTA-CCCT-
<i>Cy. colhounii</i> STE-U 705	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	GCTGC-TTGG	TGTA-CCCC-
<i>Cy. curvisporum</i> STE-U 763	GGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGTGGTTAA	AATA-XXAAA	CG-XCTCGCC
<i>Cy. curvisporum</i> STE-U 765	GGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGTGGTTAA	AATA-XXAAA	CG-XCTCGCC
<i>Cy. flexuosum</i> STE-U 2536	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. floridanum</i> ATCC 18834	GGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTGGTCAA	ATT-XGC-AA	CA-XXTCGC-
<i>Cy. floridanum</i> ATCC 18882	GGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTGGTCAA	ATTGC-XXAA	CA-XXTCGC-
<i>Cy. floridanum</i> CBS 413.67	GGAGCGCATG	AACGTTTACT	TCAACGAGGT	ATGTAGCGAA	AGAGCGCGCA	TACGCGCTCA
<i>Cy. floridanum</i> IMI 35428	GGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGTGGNTAA	AATA-XXAAA	CG-XCTCGCC
<i>Cy. floridanum</i> IMI 35429	GGAGCGCATG	AACGCTTACT	TAAACGAGGT	ATGTGGTAA	AATA-XXAAA	GCGCTC-C-
<i>Cy. floridanum</i> STE-U 2350	GGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTGGTCAA	ATT-XGC-AA	CA-XXTCGC-
<i>Cy. floridanum</i> STE-U 682	GGAGCGTATG	AACGTTTACT	TCAACGAGGT	ATGTGGTCAA	ATT-XGC-AA	CA-XXTCGC-
<i>Cy. floridanum</i> UFV 76	GGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGTGGTTAA	AATA-XXAAA	CG-XCTCGCC
<i>Cy. gracile</i> ATCC 22833	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. gracile</i> IMI 167580	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. gracile</i> PC 551197	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. gracile</i> STE-U 1586	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. gracile</i> STE-U 623	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	AGCACTCCC-
<i>Cy. graciloides</i> STE-U 1153	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACA-XGA	-GTAC-TTGC
<i>Cy. hawksworthii</i> MUCL 30866	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCACGC-GG	TGTACTCAC-
<i>Cy. heptaseptatum</i> FTCC 1002	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTT-AAAA	-CCACAC-GA	CCTACTCCC-
<i>Cy. heptaseptatum</i> FTCC 1003	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	TGTA-XAAA	ACCACAC-GA	ACTACTCCC-
<i>Cy. heptaseptatum</i> STE-U 2344	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTT-AAAA	-CCACAC-GA	CCTACTCCC-
<i>Cy. insulare</i> STE-U 616	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ATCAGC-GG	TGTACTCACA
<i>Cy. insulare</i> STE-U 768	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCGCG-GG	TGTACCACCA
<i>Cy. insulare</i> STE-U 954	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	ACCGCG-GG	TGTACTCACA
<i>Cy. leucothoes</i> ATCC 64824	CGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGT-GAAAA	-TCAGCTGA	T-AACACTC-
<i>Cy. leucothoes</i> P97.2605	CGAGCGCATG	AACGCTTACT	TCAACGAGGT	ATGT-GA-AA	ATCAGCTGA	T-AACACTC-
<i>Cy. macroconidiale</i> STE-U 307	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	CCCAT-CTGA	TCTGACCCC-
<i>Cy. macroconidiale</i> STE-U 413	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAA	CCCAT-CTGA	TCTGACCCC-
<i>Cy. mexicanum</i> STE-U 927	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTA-XAAA	ACCGTCCAA	-GAAATTTTC-
<i>Cy. mexicanum</i> STE-U 941	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTA-XAAA	ACCGTCCAA	-GAAATTTTC-
<i>Cy. multiseptatum</i> STE-U 1589	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGCG-AAA	ATCATG-XAG	TGCGCTCGC-
<i>Cy. multiseptatum</i> STE-U 1602	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGCG-AAA	ATCATG-XAG	TGCGCTCGC-
<i>Cy. naviculatum</i> STE-U 627	CGAGCGCATG	AACGCTTACT	TCAACGAGGT	AGGTAGT-GG	GCTA-GC-XA	CA-XXXCCCA
<i>Cy. naviculatum</i> STE-U 628	CGAGCGCATG	AACGCTTACT	TCAACGAGGT	AGGTAGT-GG	GCTA-GC-XA	CA-XXXCCCA
<i>Cy. ovatum</i> UFV 90	CGAGCGTATG	AACGCTTACT	TCAACGAGGT	ATGTG-XAAG	ACCACGC-GG	TGCACCCCT-
<i>Cy. parasiticum</i> ATCC 46133	GGAGCGCATG					

ATT-XCC-XX	XXXXXXXC	-G-XXXXCTC	ACACA-XXXX	XXXAC-TAGG	CCTCTGGCAA
TGTG-TCGAG	G-XCATATAA	CCCCA-XCTC	ACAC-XXXXX	GTCATGTAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	TCCGA-XCTC	ACAC-XXXXX	ATCATGTAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	TCCGA-XCTC	ACAC-XXXXX	ATCATGTAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	TCCGA-XCTC	ACAC-XXXXX	ATCATGTAGG	CTTCCGGCAA
TGTG-TCGAG	ACAACACA-X	GTAAX-XCTC	ACACAC-XXX	G-CATGTAGG	CTTCTGGCAA
TATG-TCGAG	AGA-CGCAAA	CTAAA-XCTG	ACAC-XXXXX	-XCATGTAGG	CCTTCCGGCAA
TATG-TCGAG	AGA-CGCAAA	GTAAX-XCTG	ACGC-XXXXX	-XCATGTAGG	CTTCCGGCAA
TATG-TCGAG	AGA-CGCAAA	GTAAX-XCTG	ACAC-XXXXX	-XCATGTAGG	CCTTCCGGCAA
TATG-TCGAG	AGA-CGCAAA	CTAAA-XCTG	ACAC-XXXXX	-XCATGTAGG	CCTCCTGGCAA
-XTGGTCGA-	AAAACCTGTG	CACAAA-CTC	ACAC-XXXXX	-XGATACAGG	CTTCCGGCAA
-XTGGTCGA-	AAAACCTGTG	CACAAA-CTC	ACAC-XXXXX	-XGATACAGG	CTTCCGGCAA
TT-GATCGAG	AAA-CATAAA	-CAAA-XCTC	ACAC-XXXXX	-XCATGTAGG	CTTCCGGCAA
TTTG-GCGAG	AA-XTATAGA	GCCAGCACTC	ACAC-XXXXX	-XCATCTAGG	CTTCCGGCAA
TTTG-GCGAG	AA-XTATAGA	GCCAGCACTC	ACAC-XXXXX	-XCATCTAGG	CTTCCGGCAA
CCCTG-CGAG	AAA-CAT-GA	ATAAAACTCT	ACAC-XXXXX	-XTATCAAGG	CCTNCGGCAA
-XTGGTCGA-	AAAACCTGTG	CACAAA-CTC	ACAT-XXXXX	-XGATACAGG	CTTNCGGCAA
-XTGGTCGA-	AAAACCTGTG	CACAAA-CTC	ACAT-XXXXX	-XGATACAGG	CTTCCGGCAA
TTTG-GCGAG	AA-XTATAGA	GCCAGCACTC	ACAC-XXXXX	-XCATNTAGG	CTTCCGGCAA
-XTGGG-GAG	AA-XTTTAGA	GCCAGCACTC	ACAC-XXXXX	-XCATTAGG	CTTTCGGCAA
-XTGGTCGA-	AAAACCTGTG	CACAAA-CTC	ACAC-XXXXX	-XGATACAGG	CTTCCGGCAA
TT-GATCGAG	AAG-CACAA-	GCAAA-XCTC	ACACAC-XXX	-XCATGTAGG	CTTCCGGCAA
TT-GATCGAG	AAG-CACAA-	GCAAA-XCTC	ACACAC-XXX	-XCACGTAGG	CTTCCGGCAA
TT-GATCGAG	AAG-CACAA-	GCAAA-XCTC	ACACAC-XXX	-XCATGTAGG	CTTCCGGCAA
TT-GATCGAG	AAG-CACAA-	GCAAA-XCTC	ACACAC-XXX	-XCATGTAGG	CTTCCGGCAA
TTTG-TCGGG	AAGAT-CAA-	GCGAA-XCTC	ACACAC-XXX	-XCATGTAGG	CTTCCGGCAA
-XXXGCCGAG	AGG-CACAA-	GCAAA-XCTG	ACAC-XXXXX	-XTATGTAGG	CTTCTGGCAA
TCATACCAGC	AG-XTAT-GA	GAAAA-XCTC	A-XXXXXXX	XXCATGTAGG	CCTCTGGCAA
TATATCAGC	AG-XTAT-GA	GAAAA-XCTC	A-XXXXXXX	-XXXGTGGG	CCTCTGGCAA
TCATACCAGC	CAG-TAT-GA	GAAAA-XCTC	A-XXXXXXX	XXCATGTAGG	CCTCTGGCAA
C-XXGCCGAG	AGG-CACAA-	GCAAA-XCTG	ACAC-XXXXX	-XCATGTAGG	CTTCTGGCAA
C-XGCCGAG	AGG-CACAA-	GCAAA-XCTG	ACAC-XXXXX	-XC-TGTAGG	CTTCTGGCAA
C-XXGCCGAG	AGG-CACAA-	GCAAA-XCTG	ACAC-XXXXX	-XCATGTAGG	CTTCTGGCAA
TTTG-TTG-X	AGACA-CAAA	AGCGAA-CTC	ACACAC-XXX	GTCATGTAGG	CTTNCGGCAA
TTTG-TTGAG	ACAACAAAGC	G-AA-XXCTC	ACACAC-XXX	GTCATGTAGG	CTTCCGGCAA
TTCG-TCGAT	AGG-CACAAA	GTAAX-XCTG	ACAC-XXXXX	GTCATGTAGG	CTTCCGGCAA
TTCG-TCGAT	AGG-CACAAA	GTAAX-XCTG	ACAC-XXXXX	GTCATGTAGG	CTTCCGGCAA
TTTG-TCGGG	ACG-CCCAAA	-CAAA-XCTC	ACAC-XXXXX	GTCATGTAGG	CTTCCGGCAA
TTTG-TCGGG	ACG-CCCAAA	-CAAA-XCTC	ACACAC-XXX	GTCATGTAGG	CTTCCGGCAA
TTTG-TGGAG	AAA-CATAG-	TCAAA-XCTG	ACACAC-XXX	AT-GTGTAGG	CTTCCGGCAA
TTTG-TGGAG	AAA-CATAG-	TCAAA-XCTG	ACACAC-XXX	AT-GTGTAGG	CTTCCGGCAA
TTTG-TCAAG	AAA-CATTGA	ACAAA-XCTG	ACAGACCGCA	ACCGCACAGG	CTTCCGGCAA
TTTG-TCAA-	XGAAACATTG	AACAAA-CTG	ACAGACCGCA	ACCGCACAGG	CTTCCGGCAA
TTT-GCCGAG	AAG-CACAA-	GCAAA-XCTG	ACACA-XXXX	XXCATGTAGG	CTTCCGGCAA
-XTGGTCGAA	AAA-CTTGT-	GCACAACTC	ACAC-XXXXX	-XGATACAGG	CTTCCGGCAA
-XTGGTCGAA	AAA-CTTGT-	GCACAACTC	ACAC-XXXXX	-XGATACAGG	CTTCCGGCAA
-XTGGTCGAA	AAA-CTTGT-	GCCCAACTC	ACAC-XXXXX	-XAATACAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	GCCAA-XCTC	ACAC-XXXXX	ATCATGTAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	GCCAA-XCTC	ACACA-XXXX	ATCATGTAGG	CTTCCGGCAA
TT-GACCGAG	AAG-CACAA-	GCCAA-XCTC	ACAC-XXXXX	ATCATGTAGG	CTTCCGGCAA
-XTGAT-GA-	XAAT-CTCAC	ACACG-XCT-	XXXXXXXXXX	-XXACTCAGG	CTTCCGGCAA
TT-G-CCGAG	AAG-CACAA-	GCAAA-XCTC	ACA-XXXXXX	ATCATGTAGG	CTTCCGGCAA
TT-G-CCGAG	AAG-CACAA-	GCAAA-XCTC	ACA-XXXXXX	ATCATGTAGG	CTTCCGGCAA
TTT-ACCGGG	AAG-CACAA-	GCAAA-XCTG	ACACGC-XXX	-XCGTGCAGG	CTTCTGGCAA
TTT-ACCGGG	AAG-CACAA-	GCAAA-XCTG	ACACGC-XXX	-XCGTGCAGG	CTTCTGGCAA
TTT-ACCGGG	AAG-CACAA-	GCAAA-XCTG	ACACGC-XXX	XCCGTGCAGG	CTTCTGGCAA
TTTG-CGAG	AAA-TACAG-	GCAAA-XCTG	ACACAC-XXX	AT-GTGTAGG	CTTCCGGCAA
TTTG-TCGAA	AAAGCACAA-	GCAAA-XCTG	ACACAC-XXX	-XCATGTAGG	CTTCCGGCAA
TTTG-TGAA	AAAGCACAA-	GCAAA-XCTG	ACNAC-XXX	-XCATGTAGG	CTTCCGGCAA
TTTAACCGAGC	AG-XTGCAA-	GAAAA-XCTC	A-XXXXXXX	XXCATGTAGG	CCTCTGGCAA
TTTAACCGGC	AG-XTGCAA-	GAAAA-XCTC	A-XXXXXXX	XXCATGTAGG	CCTCTGGCAA
TTTAACCGGC	AG-XTGCAA-	GAAAA-XCTC	A-XXXXXXX	XXCATGTAGG	CCTCTGGCAA
C-XXGCCGAG	AGG-CACAA-	GCAAA-XCTG	ACAC-XXXXX	-XCATGTAGG	CTTCTGGCAA
C-XGCCGAG	AGG-CACAA-	GCAAA-XCTG			

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[illegible]

Alignment 6. Part 6. ITS1 5.8S ITS2 rDNA sequence alignment from selected isolates of Hypocrealean species with nectriaceous teleomorphs and anamorphs with cylindrical macroconidia

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Ce. <i>camelliae</i> STE-U 234	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>camelliae</i> STE-U 277	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>elegans</i> STE-U 518	XXXXACATAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCATGT-XC	GTTGCCTCGG
Ce. <i>infestans</i> ATCC 44816	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-GT-XC	GTTGCCTCGG
Ce. <i>infestans</i> IMI 299376	XXXXXXAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-GT-XC	GTTGCCTCGG
Ce. <i>infestans</i> STE-U 2319	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-GT-XC	GTTGCCTCGG
Ce. <i>infestans</i> STE-U 708	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-GT-XC	GTTGCCTCGG
Ce. <i>lageniformis</i> UFV 115	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-GT-XC	GTTGCCTCGG
Ce. <i>microcylindrica</i> ATCC 38571	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>microcylindrica</i> STE-U 683	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>microcylindrica</i> STE-U 918	XXXXACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>novae-zelandiae</i> ATCC 44815	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCATGT-XC	GTTGCCTCGG
Ce. <i>parva</i> ATCC 28272	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>parva</i> STE-U 373	XXXXXXAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>peruviana</i> IMUR 1843	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Ce. <i>peruviana</i> STE-U 395	CATTACAGAG	TTTACAAC	CTCAACCCC	TGTGAAC	T	ACCATGT-XC	GTTGCCTCGG
Co. <i>destructans</i> AR 2553	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCAT-ATT	GTTGCCTCGG
Co. <i>destructans</i> CTR 71-322	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCAT-ATT	GTTGCCTCGG
Co. <i>destructans</i> var <i>coprosmae</i> CTR 73-152	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCAT-ATT	GTTGCCTCGG
Co. <i>destructans</i> var <i>coprosmae</i> GJS 85-182	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCAT-ATT	GTTGCCTCGG
Co. <i>macroconidialis</i> GJS 83-162	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCATGT-XC	GTTGCCTCGG
Cu. <i>cigneum</i> STE-U 1595	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TCAAC	GTTCCCTCGG
Cy. <i>candelabrum</i> STE-U 1674	XXXXXXAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>candelabrum</i> STE-U 1675	XXXXXXAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>floridanum</i> ATCC 18834	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>floridanum</i> ATCC 18882	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>multiseptatum</i> STE-U 1589	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>multiseptatum</i> STE-U 1602	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>scoparium</i> ATCC 38227	XXXXXXAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
Cy. <i>scoparium</i> ATCC 46300	XXXXXXAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACC-TGTTT	GTTCCCTCGG
F. <i>subglutinans</i> NRRL 22061	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	AT	ACCA-XATT	GTTGCCTCGG
Ge. <i>bulbillum</i> GJS 92-7	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	TTT	ACC-XTTTAC	GTTCCCTCGG
Gl. <i>irregularis</i> STE-U 718	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	TAT	ACC-XTTTAC	GTTGCCTCGG
Gl. <i>sumatrensis</i> STE-U 1351	CATTACCGAG	TTTACAAC	CTCAACCCC	TGTGAAC	TAT	ACC-XTTTAC	GTTGCCTCGG
X. <i>serpens</i> STE-U 1144	CATTACCGAG	TTTACAAC	CTCAACCCAA	TGTGAAC	TAT	ACC-TGTT-C	GTTCCCTCGG

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F. <i>subglutinans</i> NRRL 22061	CGG-ATCAGC	CCGC-XTCC	GGTAAACGG	GACGGCCCGC	CAGAGGACCC	C-TAAACTCT
Ce. <i>camelliae</i> STE-U 277	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>camelliae</i> STE-U 234	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>elegans</i> STE-U 518	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>infestans</i> ATCC 44816	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>infestans</i> IMI 299376	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>infestans</i> STE-U 2319	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>infestans</i> STE-U 708	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>lageniformis</i> UFV 115	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>microcylindrica</i> ATCC 38571	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>microcylindrica</i> STE-U 683	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>microcylindrica</i> STE-U 918	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>novae-zelandiae</i> ATCC 44815	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>parva</i> ATCC 28272	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>parva</i> STE-U 373	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>peruviana</i> IMUR 1843	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Ce. <i>peruviana</i> STE-U 395	CGGTGT-XXC	TTGC-TTC-X	GGC-XXXXXX	GA-XGCCCGC	CAGAGGACCC	AACAAACTCT
Co. <i>destructans</i> AR 2553	CGGTG-XXXX	CCG-TTC-X	GGC-XXXXXX	XXGCGCCCGC	CAGAGGACCC	AA-ACCCTA-X
Co. <i>destructans</i> CTR 71-322	CGGTGT-XXC	-TG-TTTC-X	GGCA-XXXXX	-XXGCGCCCGC	CAGAGGACCC	AA-ACCCTA-
Co. <i>destructans</i> var <i>coprosmae</i> CTR 73-152	CGGTGT-XXC	-TG-TTTC-X	GGCA-XXXXX	-XXGCGCCCGC	CAGAGGACCC	AA-ACCCTA-
Co. <i>destructans</i> var <i>coprosmae</i> GJS 85-182	CGGTG-XXXX	CCG-TTC-X	GGC-XXXXXX	XXGCGCCCGC	CAGAGGACTG	AA-ACCCTT-
Co. <i>macroconidialis</i> GJS 83-162	CGGTG-XXXX	CCGC-XTCC	GGCGG-XXXX	XXXXTCCCGC	CAGAGGACCC	C-CAAAACCT
Cu. <i>cigneum</i> STE-U 1595	CGGTGT-XXC	CCGCGCTCC	GGCAA-XXXX	XXGCGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>candelabrum</i> STE-U 1674	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>candelabrum</i> STE-U 1675	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>floridanum</i> ATCC 18834	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>floridanum</i> ATCC 18882	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>multiseptatum</i> STE-U 1589	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>multiseptatum</i> STE-U 1602	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>scoparium</i> ATCC 38227	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Cy. <i>scoparium</i> ATCC 46300	CGGTGT-XXX	-XXXXXXCC-	GGCAA-XXXX	XXCGGCCCGC	CAGAGGACCC	AACAAACTCT
Ge. <i>bulbillum</i> GJS 92-7	CGGCGTTC-C	CC-XXXTT-X	GG-GG-XXXX	XXTTTCCCGC	CAGAGGACCA	AACAAACCTT
Gl. <i>irregularis</i> STE-U 718	CGGCGT-XXX	CCGC-TTC-X	GGC-XXXXXX	XXGCGCCCGC	CAGAGGACCC	AA-XXACTCT
Gl. <i>sumatrensis</i> STE-U 1351	CGGCGT-XXX	CCGC-TTC-X	GGC-XXXXXX	XXGCGCCCGC	CAGAGGACCC	AA-XXACTCT
X. <i>serpens</i> STE-U 1144	CGGTGTTCGC	-TGC-TTC-X	GGCA-XXXXX	GA-GGCCCGC	CAGAGGACCC	AACAAACTCT

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[illegible][illegible]

F. subglutinans NRRL 22061
Ce. camelliae STE-U 277
Ce. camelliae STE-U 234
Ce. elegans STE-U 518
Ce. infestans ATCC 44816
Ce. infestans IMI 299376
Ce. infestans STE-U 2319
Ce. infestans STE-U 708
Ce. lageniformis UFV 115
Ce. microcylindrica ATCC 38571
Ce. microcylindrica STE-U 683
Ce. microcylindrica STE-U 918
Ce. novae-zelandiae ATCC 44815
Ce. parva ATCC 28272
Ce. parva STE-U 373
Ce. peruviana IMUR 1843
Ce. peruviana STE-U 395
Ce. destructans AR 2553
Co. destructans CTR 71-322
Co. destructans var coprosmae CTR 73-152
Co. macroconidialis GJS 83-162
Co. destructans var coprosmae GJS 85-182
Cu. cigneum STE-U 1595
Cy. candelabrum STE-U 1674
Cy. candelabrum STE-U 1675
Cy. floridanum ATCC 18834
Cy. floridanum ATCC 18882
Cy. multiseptatum STE-U 1589
Cy. multiseptatum STE-U 1602
Cy. scoparium ATCC 38227
Cy. scoparium ATCC 46300
Ge. bulbilium GJS 92-7
Gl. irregularis STE-U 718
Gl. sumatrensis STE-U 1351
X. serpens STE-U 1144

420

CTTGGTGTGTG	GGACTCGC-C-	XXXGA-XXGT	-XXXXC-AAA	T-CGC-XXGX	TTCCCCAAAT
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TTTGGTGTGTG	GAGATCGACA	-XTGAA-XG-	CCCTTCTGGG	TGTGAAGTCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGACA	-XTGA-XXGT	CCCTTC-GGG	GGCGACGCTG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGGCA	-XTGA-XXGT	-CCTTC-GGG	-GCGACGCCG	TCTCCCAAAT
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TTTGGTGTGTG	GAGATCGGCA	-XTGA-XXGT	-CCTTC-GGG	-GCGACGCCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGGCA	-XTGA-XXGT	-CCTTC-GGG	-GCGACGCCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGGCA	A-TGA-XXG-	CCCTCCGGGG	CGAAACGCCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGACA	-XTGAA-XG-	CCCTTCTGGG	TGTGAAGTCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGACA	-XTGAA-XG-	CCCTTCTGGG	TGTGAAGTCG	TCTCCCAAAT
TTTGGTGTGTG	GAGATCGACA	-XTGAA-XG-	CCCTTCTGGG	TGTGAAGTCG	TCTCCCAAAT
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TTTGGTGTGTG	GAGATCGGCA	-XTGA-XXGT	-CCTTC-GGG	-GCGACGCCG	TCTCCCAAAT
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TTTGGTGTGTG	GAGATCGACA	-XTGAA-XG-	CCCTTCTGGG	TGTGAAGTCG	TCTCCCAAAT
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CTTGGTGTGTG	GAGATCGGC-	XXXGA-XXG-	CCCT-CCGGG	G-CGC-GCCG	TCTCCCAAAT
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-TTGGTGTGTG	GGGATCGGCA	-XXXAGGCG-	ACCTTC-GGG	-GCCG-GCCG	TCCCTAAAT

F. subglutinans NRRL 22061
Ce. camelliae STE-U 277
Ce. camelliae STE-U 234
Ce. elegans STE-U 518
Ce. infestans ATCC 44816
Ce. infestans IMI 299376
Ce. infestans STE-U 2319
Ce. infestans STE-U 708
Ce. lageniformis UFV 115
Ce. microcylindrica ATCC 38571
Ce. microcylindrica STE-U 683
Ce. microcylindrica STE-U 918
Ce. novae-zelandiae ATCC 44815
Ce. parva ATCC 28272
Ce. parva STE-U 373
Ce. peruviana IMUR 1843
Ce. peruviana STE-U 395
Co. destructans AR 2553
Co. destructans CTR 71-322
Co. destructans var coprosmae CTR 73-152
Co. destructans var coprosmae GJS 85-182
Co. macroconidialis GJS 83-162
Cu. cigneum STE-U 1595
Cy. candelabrum STE-U 1674
Cy. candelabrum STE-U 1675
Cy. floridanum ATCC 18834
Cy. floridanum ATCC 18882
Cy. multiseptatum STE-U 1589
Cy. multiseptatum STE-U 1602
Cy. scoparium ATCC 38227
Cy. scoparium ATCC 46300
Ge. bulbilium GJS 92-7
Gl. irregularis STE-U 718
Gl. sumatrensis STE-U 1351
X. serpens STE-U 1144

[illegible]

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A-GCAGCAAG	CCCACGCCGT	TAAACCCCCC	ACTTT-XXXX	TGA-XXGTTT	GACCTCGAAT
AA-CAGCGTG	CCCACGCCGT	TAAACCCCCC	ACTTT-XXXX	TGAA-XGTTT	GACCTCGAAT
A-GCAGCGCG	GCCACGCCGT	TAAACCCCCA	ACTTTT-XXC	TGA-XXGTTT	GACCTCGAAT
A-GCAGCGCG	GC-ACGCCGT	TAAACCCCCA	ACTTTT-XXC	TGA-XXGTTT	GACCTCGAAT
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A-GCAGCGCG	GCCACGCCGT	TAAACCCCCA	ACTTTT-XXA	KKA-XXGTTT	KACCTCGAAT
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A-GCAGCAAG	CCCACGCCGT	TAAACCCCCC	ACTTT-XXXX	TGA-XXGTTT	GACCTCGAAT
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A-XCAGCGTG	ACCACGCCGT	AAAACCCCCC	ACTT-XXXXX	TGAAAGGTT-	GACCTCGGAT
A-XCAGCGTG	GCCACGCCGT	AAAACCCCCC	ACTT-XXXXX	TGAAAGGTT-	GACCTCGGAT
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A-XCAGCGTG	GCCACGCCGT	AAAACCCCCC	ACTT-XXXXX	TGAAAGGTT-	GACCTCGGAT
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GCTCGGCGCG	GCCAAAGCGT	TAAACCCCCA	ACTTTTTTTX	XXXXXXXXXX	XXXXXXXXXX
TCTCGGTGCG	ACCACGCCGT	AAAACCCCCA	ACTTTTT-XC	TG-XXGXGX	XXXXXXXXXX
TCTCGGTGCG	ACCACGCCGT	AAAACCCCCA	ACTTTTT-XC	TG-XXGXGX	XXXXXXXXXX
TCTCGGTGCG	ACCACGCCGT	AAAACCCCCA	ACTTTTT-XC	TG-XXGXTT	GACCTCGAAT
TCTCGGTGCG	ACCACGCCGT	AAAACCCCCA	ACTTTTT-XC	TG-XXGXTT	GACCTCGAAT
TCTCGGTGCG	GCCACGCCGT	AAAAACCCCA	ACTTTTTT-C	TG-XXGXTT	GACCTCGAAT
TCTCGGTGCG	GCCACGCCGT	AAAAACCCCA	ACTTTTTT-C	TG-XXGXTT	GACCTCGAAT
TCTCGGTGCG	GCCACGCCGT	AAAAACCCCA	ACTTTTTT-C	TG-XXXXXX	XXXXXXXXXX
-CGCGGCGCG	GCCAAAGCGT	TAAACCCCCA	ACTT-XXXXX	TGAA-XGTTT	GACCTCGGAT
-CGCGGCGCG	GCCACGCCGT	TAAACCCCCC	ACTT-XXXXX	TGAA-GGTT-	GACCTCGGAT
-CGCGGCGCG	GCCACGCCGT	TAAACCCCCC	ACTT-XXXXX	TGAA-GGTT-	GACCTCGGAT
ACGCGGCGCG	GCCAAAGCGT	TAAACCCCCA	ACTTTTTTTT	TG-XXXXTT	GACCTCGAAT

564
 CAGGTAGGAA TACCCGCTGA ACXX
 CAGGTAGGAT TACCCGCTXX XXXX
 CXXXXXXXXX XXXXXXXXXXXX XXXX
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 CAGGTAGGAT TACCCGCTGA ACTT
 CAGGTAGGAT TAXXXXXXXXXX XXXX
 CAGGTAGGAT TACCCGCTGX XXXX
 CAGGTAGGAT TACCCGCTGA AXXX
 CAGGTAGGAT TACCCGCTGA ACTT
 CAGGTAGGAT TACCCGCTGA ACTT
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 CAGGTAGGAT TAXXXXXXXXXX XXXX
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 CAGGTAGGAC TACCCGCTGA ACTT

Alignment 7. Part 6. ITS1 5.8S ITS2 rDNA sequence alignment from isolates of selected *Cylindrocladiella* species

		60	
<i>F. subglutinans</i> NRRL 22061	CATTACCGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CAATT-GTTG CCTCGGCGG-
<i>Ce. camelliae</i> STE-U_234	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. camelliae</i> STE-U_277	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. elegans</i> STE-U_518	MTCNACATAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CATGTCGTTG CCTCGGCGGT
<i>Ce. infestans</i> ATCC 44816	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CA-GTCGTTG CCTCGGCGGT
<i>Ce. infestans</i> IMI_299376	XXXXXXAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CA-GTCGTTG CCTCGGCGGT
<i>Ce. infestans</i> STE-U_2319	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CA-GTCGTTG CCTCGGCGGT
<i>Ce. infestans</i> STE-U_708	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CA-GTCGTTG CCTCGGCGGT
<i>Ce. lageniformis</i> UVF_115	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CA-GTCGTTG CCTCGGCGGT
<i>Ce. microcylindrica</i> ATCC 38571	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. microcylindrica</i> STE-U_683	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. microcylindrica</i> STE-U_918	XXXXXACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. novae-zelandiae</i> ATCC 44815	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACATAC CATGTCGTTG CCTCGGCGGT
<i>Ce. parva</i> ATCC 28272	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. parva</i> STE-U_373	XXXXXXXXXAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. peruviana</i> IMUR 1843	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
<i>Ce. peruviana</i> STE-U_395	CATTACAGAG	TTTACAACCTC	CCAAACCCCT GTGAACCTTAC CATGTCGTTG CCTCGGCGGT
		120	
<i>F. subglutinans</i> NRRL 22061	ATCAGCCCCG	TCCCGGTAAA	ACGGGACGGC CCGCCAGAGG ACCCC-TAAA CTCT-GTTTC
<i>Ce. camelliae</i> STE-U_234	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. camelliae</i> STE-U_277	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. elegans</i> STE-U_518	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. infestans</i> ATCC 44816	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. infestans</i> IMI_299376	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. infestans</i> STE-U_2319	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. infestans</i> STE-U_708	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. lageniformis</i> UVF_115	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. microcylindrica</i> ATCC 38571	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. microcylindrica</i> STE-U_683	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. microcylindrica</i> STE-U_918	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. novae-zelandiae</i> ATCC 44815	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. parva</i> ATCC 28272	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. parva</i> STE-U_373	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. peruviana</i> IMUR 1843	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
<i>Ce. peruviana</i> STE-U_395	GT-XXCTTGC	TTCGGC-XXX	-XXXGA-XGC CCGCCAGAGG ACCCAACAAA CTCTTGTTTT
		180	
<i>F. subglutinans</i> NRRL 22061	-XXTATATGT	AACCTCTGAG	TAA-AACCAT AAATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. camelliae</i> STE-U_234	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. camelliae</i> STE-U_277	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. elegans</i> STE-U_518	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. infestans</i> ATCC 44816	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. infestans</i> IMI_299376	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. infestans</i> STE-U_2319	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. infestans</i> STE-U_708	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. lageniformis</i> UVF_115	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. microcylindrica</i> ATCC 38571	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. microcylindrica</i> STE-U_683	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. microcylindrica</i> STE-U_918	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. novae-zelandiae</i> ATCC 44815	TTTTAGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. parva</i> ATCC 28272	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. parva</i> STE-U_373	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. peruviana</i> IMUR 1843	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
<i>Ce. peruviana</i> STE-U_395	TTT-AGTATT	ATCT-XXGAG	TGACAAGTTT -AATAAATCA AAACCTTTCAA CAACGGATCT
		240	
<i>F. subglutinans</i> NRRL 22061	CTTGTTCTG	GCATCGATGA	AGAACGCAGC AAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. camelliae</i> STE-U_234	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. camelliae</i> STE-U_277	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. elegans</i> STE-U_518	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. infestans</i> ATCC 44816	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. infestans</i> IMI_299376	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. infestans</i> STE-U_2319	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. infestans</i> STE-U_708	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. lageniformis</i> UVF_115	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. microcylindrica</i> ATCC 38571	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. microcylindrica</i> STE-U_683	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. microcylindrica</i> STE-U_918	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. novae-zelandiae</i> ATCC 44815	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. parva</i> ATCC 28272	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. parva</i> STE-U_373	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. peruviana</i> IMUR 1843	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA
<i>Ce. peruviana</i> STE-U_395	CTTGTTCTG	GCATCGATGA	AGAACGCAGC GAAATGCGAT AAGTAATGTG AATTGCAGAA

[illegible][illegible]

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AAG-CCCTTC TGGGTGTGAA GTCGTCTCCC AAATATAGTG GCGGCTCTCG TGTAGCTTCC
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A-GTCCCTTC -GGGGGCGAC GTCGTCTCCC AAATATAGTG GCGGCTCTCG TGTAGCTTCN
A-GT-CCCTC -GGG-GCGAC GCGGTCTCCC AAATATAGTG GCGGCTCTCG TGTAGCTTCC
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TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC
TATGCGGTAG	TAGCACA-XC	CTCGC-ACTG	GAAACAGCG	TGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCG	CGGCCACGCC	GTTAAACCCC
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TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCG	CGGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAACAGCT	TGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAACAGCG	TGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAACAGCG	TGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC
TATGC-GTAG	TAGCACA-XC	CTCGC-ACTG	GAAAGCAGCA	AGCCACGCC	GTTAAACCCC

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<i>F. subglutinans</i> NRRL 22061	-AACTT-XCT	GAATGTT-GA	CCTCGGATCA	GGTAGGAATA	CCCGCTGAAC	XX
<i>Ce. camelliae</i> STE-U_234	CCACTTT-CT	GA-XGTTTGA	CCTCGACTCX	XXXXXXXXXX	XXXXXXXXXX	XX
<i>Ce. camelliae</i> STE-U_277	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCXXXXX	XX
<i>Ce. elegans</i> STE-U_518	CAACTTT-CT	GAA-GTTTGN	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. infestans</i> ATCC_44816	CAACTTTTCT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. infestans</i> IMI_299376	CAACTTTTCT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. infestans</i> STE-U_2319	CAACTTTTCT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. infestans</i> STE-U_708	CAACTTTTAK	KA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. lageniformis</i> UFV_115	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	XXXXXXXXXX	XX
<i>Ce. microcylindrica</i> ATCC_38571	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGXXX	XX
<i>Ce. microcylindrica</i> STE-U_683	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAX	XX
<i>Ce. microcylindrica</i> STE-U_918	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. novae-zelandiae</i> ATCC_44815	CAACTTT-CT	GAA-GTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. parva</i> ATCC_28272	CAACTTT-CT	GAA-GTTTGA	CCTTGAATCA	XXXXXXXXXX	XXXXXXXXXX	XX
<i>Ce. parva</i> STE-U_373	CAACTTT-CT	GAA-GTTTGA	CCTCGAATCA	GGTAGGATTA	CCCGCTGAAC	TT
<i>Ce. peruviana</i> IMUR_1843	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	XXXXXXXXXX	XX
<i>Ce. peruviana</i> STE-U_395	CCACTTT-CT	GA-XGTTTGA	CCTCGAATCA	GGTAGGATTA	CCXXXXXXXX	XX

Alignment 8. Part 6. 5' end of β -tubulin gene DNA sequence alignment of *Cylindrocladiella* species

<i>F. subglutininans</i> _NRRL_22061	GCGTTGAGTT	TATGGTGCCC	CTGATTCTAC	CCCGCTGGGC	GGTGG-XCA-	XXXXXGC-TC
Ce._camelliae STE-U_234	GCGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCCTC
Ce._camelliae STE-U_277	GCGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCTC
Ce._elegans STE-U_518	GCGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	C-XXXGCCTC
Ce._infestans ATCC_44816	XXXXXXXXXX	-XXGTGCCCC	CTSATTCTAT	CCMGCCGAAT	CGTTTTCCAC	CCACCGCCTC
Ce._infestans IMI_299376	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	CCACCGCCTC
Ce._infestans STE-U_2319	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	CCACCGCCTC
Ce._infestans STE-U_708	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	CCACCGCCTC
Ce._lageniformis UFV_115	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	C-XXXGCCTC
Ce._microcylindrica ATCC_38571	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	CATTITCCAC	C-XXXGCYTC
Ce._microcylindrica STE-U_683	GCGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCCTC
Ce._microcylindrica STE-U_918	GCGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCCTC
Ce._novae-zelandiae ATCC_44815	XXXXXXXXXX	XXXXXTGCCC	CTGATTCTAC	CCCGCGCAAY	CGTTTTCCAC	C-XXXGCCTC
Ce._parva ATCC_28272	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	C-XXXGCTTC
Ce._parva STE-U_373	GCGTT-XGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAT	CGTTTTCCAC	C-XXXGCTTC
Ce._peruviana IMUR_1843	GCGTT-TGTT	-XXGN TGCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCCTC
Ce._peruviana STE-U_395	ACGTT-TGTT	-XXGTGCCCC	CTGATTCTAC	CCCGCGCAAC	CATTITCCAC	C-XXXGCCTC

	120
<i>F. subglutini</i> NRRL 22061	AACGACAATG CACGATAGCT AGCA-GCTTT A-XAATACCT TCTGTCAAG- ATGAAGAAGC
<i>Ce. camelliae</i> STE-U_234	GACAACAA-C AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATGTCTTGA ATGAGATTGC
<i>Ce. camelliae</i> STE-U_277	GACAACAA-C AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATGTCTTGA ATGAGATTGC
<i>Ce. elegans</i> STE-U_518	GACAACAA-C AAAGTTCGGG ATTATGCCCA C-XXXGTCGT GATA-GTTGG ATCAGATTGC
<i>Ce. infestans</i> ATCC_44816	GACAACAA-C AAAGTTCGGG AT-XXGCCCA CCCACATCGT GATATCT-GA AGACAATGGC
<i>Ce. infestans</i> IMI_299376	GACAACAA-C AAAGTTCGGG AT-XXGCCCA CCCACATCGT GATATCT-GA AGACAATGGC
<i>Ce. infestans</i> STE-U_2319	GACAACAA-C AAAGTTCGGG AT-XXGTCCA CCCACATCGT GATATCT-GA AGACAATGGC
<i>Ce. infestans</i> STE-U_708	GACAACAA-C AAAGTTCGGG AT-XXGTCCA CCCACATCGT GATATCT-GA AGACAATGGC
<i>Ce. lageniformis</i> UFV_115	GACAACAA-C AAAGTTCGGG AT-XXGCCCA CCCACACCAT GATATCT-GA ACATAATGGC
<i>Ce. microcylindrica</i> ATCC_38571	GGCAACAACAA-AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATATCTTGA ATGAGATTGC
<i>Ce. microcylindrica</i> STE-U_683	GACAACAA-C AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATATCTTGA ATGAGATTGC
<i>Ce. microcylindrica</i> STE-U_918	GACAACAA-C AAAGTTCGCA ATAATGCCCA C-XXXGTCGT GATATCTTGA ATCAGATTGC
<i>Ce. novae-zelandiae</i> ATCC_44815	GACAACAA-C AAAGTTCGGG ATTATGCCCA C-XXXGTCGT GATA-CTTGG ATCAGATTGC
<i>Ce. parva</i> ATCC_28272	GACAACAA-C AAAGTTCGGG ATGATACCCA C-CAC-XCGT AATATCT-GG ATACAATGGC
<i>Ce. parva</i> STE-U_373	GACAACAA-C AAAGTTCGGG ATGATACCCA C-CAC-XCGT GATATCT-GG ATACAATGGC
<i>Ce. peruviana</i> IMUR_1843	GACAA-XXXX AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATATCTTGA ATCAGATTGC
<i>Ce. peruviana</i> STE-U_395	GACAA-XXXX AAAGTTCGGG ATAATGCCCA C-XXXGTCGT GATATCTTGA ATCAGATTGC

	180
F. subglutinians NRRL 22061	TAATCAGAT- CTTTTCTCT- GCGATAGGTT CACCTCCAGA CCGGTCA GTT CGTAAGTGCT
Ce. camelliae STE-U_234	TAATT-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA
Ce. camelliae STE-U_277	TAATT-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA
Ce. elegans STE-U_518	TAATT-XATG TGTTCCTG-A AATATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTACA
Ce. infestans ATCC 44816	TAATTTTGTG TGTTCCTGCG AATATAGGTC CACCTCCAGA CCGGTCA GTT CGTAAGTACA
Ce. infestans IMI_299376	TAATTTTGTG TGTTCCTGCG AATATAGGTC CACCTCCAGA CCGGTCA GTT CGTAAGTACA
Ce. infestans STE-U_2319	TAATTTTGTG TGTTCCTGCG AATATAGGTC CACCTCCAGA CCGGTCA GTT CGTAAGTACA
Ce. infestans STE-U_708	TAATTTTGTG TGTTCCTGCG AATATAGGTC CACCTCCAGA CCGGTCA GTT CGTAAGTACA
Ce. lageniformis UFV_115	TAATTTTGTG TGTTCCTGCG AATATAGGTC CACCTCCAGA CCGGTCA GTT CGTAAGTACA
Ce. microcylindrica ATCC_38571	TAATT-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG NGTAAGTTTA
Ce. microcylindrica STE-U_683	TAATC-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA
Ce. microcylindrica STE-U_918	TAATC-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA
Ce. novae-zelandiae ATCC_44815	TAATT-XATG TGTTCCTG-A AATATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTACA
Ce. parva ATCC_28272	TAATT-XCTC TGTTCCTCAA AATATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTACA
Ce. parva STE-U_373	TAATT-XCTC TGTTCCTCAA AATATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTACA
Ce. peruviana IMUR_1843	TAATC-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA
Ce. peruviana STE-U_395	TAATC-XATG TGTTCCTG-A ACTATAGGTC CACCTCCAGA CCGGCCAGTG CGTAAGTTTA

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F. subglutininans NRRL 22061	CATCGCTTCC TCG-ACGTCG CATGTGGGGG ATGCTCAGCA TG-TTTATCA GGGTAACCAA
Ce. camelliae STE-U_234	CACC TCACCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA
Ce. camelliae STE-U_277	CACCTCACCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA
Ce. elegans STE-U_518	TCCC GAACCT CGACAGGCT T-GCGGGCAT TTGCTAACGG TGCCATAATA GGGTAACCAA
Ce. infestans ATCC 44816	TTCTCTCACCT GCACAAGCCT C-GTCAACGG CTGCTAACGG TGTCTGCATA GGGTAACCAA
Ce. infestans IMI 299376	TTCTCTCACCT CGACAAGCCT C-GTCAACGG CTGCTAACGG TGTCTGCATA GGGTAACCAA
Ce. infestans STE-U_2319	TTCTCTCACCT CGACAAGCT C-GTCAACGG CTGCTAACGG TGTCTGCATA GGGTAACCAA
Ce. infestans STE-U_708	TTCTCTCACCT GCACAAGCT C-GTCAACGG CTGCTAACGG TGTCTGCATA GGGTAACCAA
Ce. lageniformis UFV 115	TTCTCTCACCT CGACAAGCCT C-GTCAACGG CTGCTAACGG TGTCTTGATA GGGTAACCAA
Ce. microcylindrica ATCC 38571	CACCTCACCT TCACGAGTCT C-TGCCGGCT TTGCTCNCA TT-CATAACA GGGTAACCAA
Ce. microcylindrica STE-U_683	CACCTTGCCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA
Ce. microcylindrica STE-U_918	CACCTTGCCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA
Ce. novae-zelandiae ATCC 44815	TTC -GAACCT CGACGAACCT T-GCGGGCAT TTGCTAACGG TGGCATAATA GGGTAACCAA
Ce. parva ATCC 28272	TTCTCTCACCT CGATAGGCCT C-AACGGCGG GTGCTAACGG TTTCTCAATA GGGTAACCAA
Ce. parva STE-U_373	TTCTCTCACCT CGATAGGCCT C-AACGGCGG GTGCTAACGG TTTCTCAATA GGGTAACCAA
Ce. peruviana IMUR 1843	CACCTTGCCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA
Ce. peruviana STE-U_395	CACCTTGCCT TCACGAGTCT C-TGCCGGCT TTGCTCACGA TA-CATAACA GGGTAACCAA

300

360

420

480

532

<i>F. subglutinans</i> NRRL 22061	ACGCC-GTCC -GAGCTGGTC CCTTCGGTCA NGCTCTTCCG TCCCGACAAC TT
<i>Ce. camelliae</i> STE-U_234	ACGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTTCG CCCCAGACAAC TT
<i>Ce. camelliae</i> STE-U_277	ACGCC-GTCC -GTGCCGGTC CTTTCGGTCA AGCTCTTCCG CCCAGACAAC TT
<i>Ce. elegans</i> STE-U_518	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTTCG CCCAGACAAC TT
<i>Ce. infestans</i> ATCC_44816	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTTCG TCCCGACAAC TT
<i>Ce. infestans</i> IMI_299376	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA AGCTCTTCCG TCCCGACAAC TT
<i>Ce. infestans</i> STE-U_2319	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTTCG CCCAGACAAC TT
<i>Ce. infestans</i> STE-U_708	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA AGCTCTTTCG CCCCAGACAAC TT
<i>Ce. lageniformis</i> UFV_115	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTTCG CCCCAGACAAC TT
<i>Ce. microcylindrica</i> ATCC_38571	ACGCC-GTCC -GTGCCGGTC CTTTXXXXXX XXXXXXXXXX XXXXXXXXXX XX
<i>Ce. microcylindrica</i> STE-U_683	ACGCC-GTCC -GTGCTGGTC CTTTCGGTCA AGCTCTTCCG CCCCAGACAAC TT
<i>Ce. microcylindrica</i> STE-U_918	XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XX
<i>Ce. novae-zelandiae</i> ATCC_44815	ACGCC-GTCC CGTGCCGGTC CTTTCGGTCA AGCTCTTCCG CCCCAGACAAC TT
<i>Ce. parva</i> ATCC_28272	ATGCCCGTCC -GTGCCGGTC CTTTGGTCA AGCTNTTTCG CCCCAGACAAC TT
<i>Ce. parva</i> STE-U_373	ATGCC-GTCC -GTGCCGGTC CTTTCGGTCA NGCTCTTCCG CCCCAGACAAC TT
<i>Ce. peruviana</i> IMUR_1843	ACGCC-GTCC -GTGCTGGTC CTTTCGGTCA NGCTCTTCCG CCCCAGACAAC XX
<i>Ce. peruviana</i> STE-U_395	ACGCC-GTCC -GTGCTGGTC CTTTCGGTCA NGCTCTTCCG CCCCAGACAAC TT